



US007455995B2

(12) **United States Patent**
Tanner et al.

(10) **Patent No.:** **US 7,455,995 B2**
(45) **Date of Patent:** **Nov. 25, 2008**

(54) **BAALC EXPRESSION AS A DIAGNOSTIC
MARKER FOR ACUTE LEUKEMIA**

(75) Inventors: **Stephan Markus Tanner**, Columbus,
OH (US); **Albert de la Chapelle**,
Delaware, OH (US)

(73) Assignee: **The Ohio State University Research
Foundation**, Columbus, OH (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 705 days.

(21) Appl. No.: **10/293,239**

(22) Filed: **Nov. 12, 2002**

(65) **Prior Publication Data**

US 2003/0119043 A1 Jun. 26, 2003

Related U.S. Application Data

(60) Provisional application No. 60/348,210, filed on Nov.
9, 2001.

(51) **Int. Cl.**
C12P 19/34 (2006.01)
C12Q 1/68 (2006.01)

(52) **U.S. Cl.** **435/91.2**; 435/91.21; 435/6

(58) **Field of Classification Search** 435/6
See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO WO 03/040347 5/2003

OTHER PUBLICATIONS

International Search Report dated Aug. 23, 2004.

“Involvement of BAALC, a novel gene, in hematopoiesis and acute
leukemia” by Tanner, et al., *Blood*, (Nov. 16, 2001) vol. 98, No. 11,
Part 1, pp. 800a-801a.

“Overexpression of BAALC suppresses normal hematopoiesis” by
Baldus, et al., *Blood*, vol. 100, No. 11, Abstract No. 4147, Nov. 16,
2001.

“BAALC, the human member of a novel mammalian neuroectoderm
gene lineage, is implicated in hematopoiesis and acute leukemia” by
Tanner, et al., *PNAS*, Nov. 20, 2001, vol. 98, No. 24, pp. 13901-
13906.

Abstract P-300, “Gene expression in acute myeloid leukemia with
trisomy 8” by Tanner, et al., 21st Annual Meeting of the European
Society of Human Genetics, Geneva, Switzerland, May 29-Jun. 1,
1999.

Abstract 18434, “Novel gene in Acute Myeloid Leukemia with
Trisomy 8” by Tanner, et al, *The American Journal of Human Genet-
ics*, vol. 65, No. 4, Oct. 1999.

Abstract 3330, “Involvement of BAALC, a Novel Human Gene, in
Hematopoiesis and Acute Leukemia” by Tanner, et al., Forty-third
Annual Meeting of the American Society of Hematology, Orlando,
Florida, Dec. 7-11, 2001.

Abstract LB-138, “Expression of BAALC predicts adverse prognosis
in adult *de novo* acute myeloid leukemia (AML) with normal
cytogenetics: A Cancer and Leukemia Group B (CALGB) Study” by
Tanner, et al., 93rd Annual Meeting of the American Association for
Cancer Research, San Francisco, California, Apr. 6-10, 2002.

GenBank Accession No. AA400649 (submitted Apr. 28, 1997).

GenBank Accession No. AC025370 (submitted Feb. 20, 2002).

GenBank Accession No. AC025936 (submitted May 27, 2001).

GenBank Accession No. AF363578 (submitted Mar. 22, 2001).

GenBank Accession No. AF371319 (submitted Apr. 17, 2001).

GenBank Accession No. AF371320 (submitted Apr. 17, 2001).

GenBank Accession No. AF371321 (submitted Apr. 17, 2001).

GenBank Accession No. AF371322 (submitted Apr. 17, 2001).

GenBank Accession No. AF371323 (submitted Apr. 17, 2001).

GenBank Accession No. AF371324 (submitted Apr. 17, 2001).

GenBank Accession No. AF371325 (submitted Apr. 17, 2001).

GenBank Accession No. AF371326 (submitted Apr. 17, 2001).

GenBank Accession No. BF 190130 (submitted Nov. 2, 2000).

GenBank Accession No. BF192691 (submitted Nov. 2, 2000).

GenBank Accession No. AB073318 (submitted Oct. 19, 2001).

Wang et al., “BAALC 1-6-8 protein is targeted to postsynaptic lipid
rafts by its N-terminal myristoylation and palmitoylation, and inter-
acts with α , but not β , subunit of Ca²⁺/calmodulin-dependent protein
kinase II,” *J. of Neurochemistry*, 92, 647-659 (2005).

Primary Examiner—Gary Benzion

Assistant Examiner—Cynthia Wilder

(74) *Attorney, Agent, or Firm*—Calfee, Halter & Griswold
LLP

(57) **ABSTRACT**

Overexpression of the gene, BAALC, in biological samples
from a patient is prognostic for tumor aggressiveness and
unfavorable patient outcome. The present invention provides
polynucleotide primers and probes for assaying for overex-
pression of BAALC transcripts. Kits containing the primers
and probes are also provided. Also provided are antibodies for
assaying for overexpression of BAALC proteins as well as
peptide immunogens for producing the anti-BAALC antibod-
ies. The present invention also provides methods for charac-
terizing acute myelogenous leukemia, chronic myelogenous
leukemia and prostate cancer in a patient, base on detection of
BAALC overexpression.

11 Claims, 16 Drawing Sheets

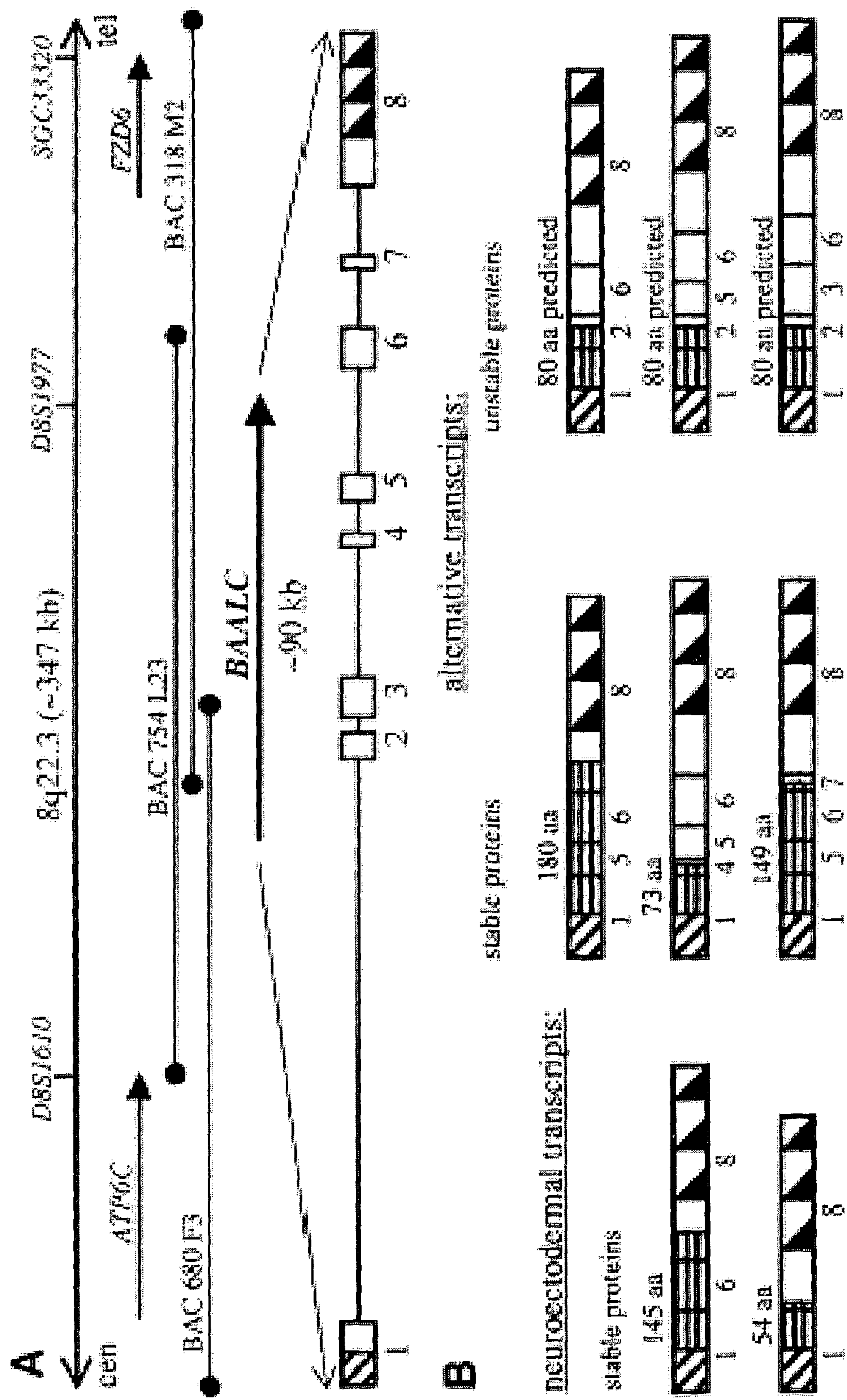


Fig. 1

BAALC human exons:

Exon 1

gagagggcccgactagggggcgccggggcaccgcaggagctccgcgcgggtgcagcgcggggcgaggcggggacgcgatgt
cgccgcgcgcgcctccttgccggggcggggctgcgcctccggggctgagccgcgcgcagagccgacagccgagcagccgct
gggcgcctccgcgcggcgaggaggATGGGCTGCGGCGGGAGCCGGGCGGATGCCATCGAGCCCCGCTACTACGAGAGCTGG
ACCCGGGAGACAGAATCCACCTGGCTCACCTACACCGACTCGGACGCGCCGCCAGCGCCGCCGCSCCGGACAGCGGCC
CGAAGCGGGCGGCCTGCACTCGG

Exon 2

CCCATTACCTCTTGCCTTTGCACTTGCCTGGAGAGACAACAGTTTAGGGGCTCTGCTGGTTCAAGAAGGACTGTGCAGG
TAGCATGGCCACACACCATGTACAG

Exon 3

GTTCTGGTGCTTAGGAGTGGACATCTTTGGGACCGAGGGTTATTCTGCCTTCTTACCATGTCACCAGAGTTGTGCTAATA
CACAGAGAGCTTCAGGGGATGAGATCTGCCATTTCATTGAGCACCTTCTGTGCGGCAGACAGTGTTAG

Exon 4

GGTGCCTTGAGGAACATTACCATCTGACTGCCCTACAGAAAGTTGGGCATCCCAACCATTGATTTAAAAAG

Exon 5

TTCTGGAGGCTGAGAAGTCCAAGATCAAGGCACCAACAGATTTCAGTGTCTGATGAAGGCTTGTTCTCTGCTTCAAAGATG
GCACCTCTTGCTGTGTTCTCACATG

Exon 6

GCATGCTGGAAGATGGACTGCCCTCCAATGGTGTGCCCCGATCTACAGCCCCAGGTGGAATACCCAACCCAGAGAAGAAG
ACGAAGTGTGAGACCCAGTGCCTCAATCCCAGAGCCTCAGCTCAGGCCCTCTGACCCAGAAACAGAATGGCCTTCAGAC
CACAGAG

Exon 7

GTTCTGCTGCCTTCTTAATGTCTGATCTTCTATCTGAAGTTCAAGTTGAAAAGCAACAACCTCCTTTTGGCACTCGATACA
AACTCCAGC

Exon 8

GCTAAAAGAGATGCTAAGAGAATGCCTGCAAAAGAAGTCACCATTAATGTAACAGATAGCATCCAACAGATGGACAGAAG
TCGAAGAATCACAAGAAGTGTGTCACTAGcagagagtcgaagcagaagggcagatggacttcttcagtgtccttcacg
gcactggatcccatcaaagaacctgaagaagtggctgccccttgctggacctgaattctactgagtccttggaagact
gtcttacctggcagcaaaactgctgctgatttgggtgggaccttctgagccttctacttatcatgtaaatgtattggcaca
gtgcttacatatgttAATAAActgcaaatgtgcagttcagtttgtctctttgcaactcctgtaatacgggtctggtgtaaa
agtagtgagttaaagctacaggtcagtttatgaaacagaaaagtaggaatgcattttctgggtgaaagagtcacacctta
gtgctataactctcctgcccagatagtgattctgtttcaggcaagcttattctttccttctttcatttttaaatattgt
cattacaaatcttaccaggttcacttaaaagctggcttccatccaactctaaacccacatattgaaaaaatcaaggtaca
ggaaaactccttggttatccttggttcccttagcttggtatgagacagatcggatccagtttcccatgcaccaacccactgc
ccatggcatgtctttgggaggtgtctgtgaagcagtcatacctgctcctcatctgcctggaaagtcctcctattccagt
tccatgttggcctccagtccttaattgtcaccatgcttgtggccaatgcaccaaataaggataccctcagggctcagct
agacattgcaattttgcatagctttccagttccctttgcttgtctcttgactgtcttccctctctatcggggtcacttg
caattgttaatacaagattgaacactgcgtaggagagggagatgatccagagacatgtggcagcagggcatggcttccct
tggcctctctgtacactgcccaggactgtcattttggcatctgcaaaggaatcactttagaaagccagcacctgggtga
tgtgtattcactgacattagattgatgtgactgcattagaaatgaggtagctgacacagaaaaaggatgttttgata
ggaataattttctagtatgtcttgaaacatgttcatctggaagtattttcctccaaagtaatgtagcatgatttttcaag
gattgttaacatgcctgggattgggaaagataggactaaagttgtgcccactatataATAAAattccatgttttagcaga
aataggcagcctattgggtgttatgtttatgtaacatagtcagagaaactgacatgcaggtcaaaagtcagatacgaacc
tccttatctgctaactctgttattcttcaaacacaacgtgggtagtgtcatttttccctccttccctccattggcagatt
gtatattttattcacaaaacattaaatgtccatcctgtgccaggtactatgcagatgttgagggatttggggctctggttag
tcgtgactatctatcctgaatctaacagtgacttcataactaggagactgaattagacccttaaggatatgtgtgttg
caaatcactctgcaatggaaacttttatattcagggtagggttgtgtcttaaactagggtgttctaatcaatgtacaagac
tttaccatacacgcaactatagtttttctaaaccttcatttttgtgattctttgagaaagggcttttaggaactttat
gttctaaaaaatgtttttaacaataataagataaaagaaaaacctgtgattcatatgtcccactggcattactcagcag
gagccccagctgcccaggttggcagtgatcctgcaagttcaagggtctttctccctggggatgtgctttgtggcttc
tctttacagctttgtttctgcatcagttcactgctgcatgtttgtttggaatttatcaccttaagaaagtgtctctgtttt
atatagaaacactttctcacttacaggggagaaaggaatgcagggcacatgatctggccctcccccagaaacatctggatt
tcacggagacagcaaccagaagttaaaccatgtgactaaaaatgcactctggctactttttcatgtatgtatgagacagaa
actaatccttactatcctattaggataccacttttcattgcaagtttgtgtcATAAAgtcattaattttaaacat

Fig. 2

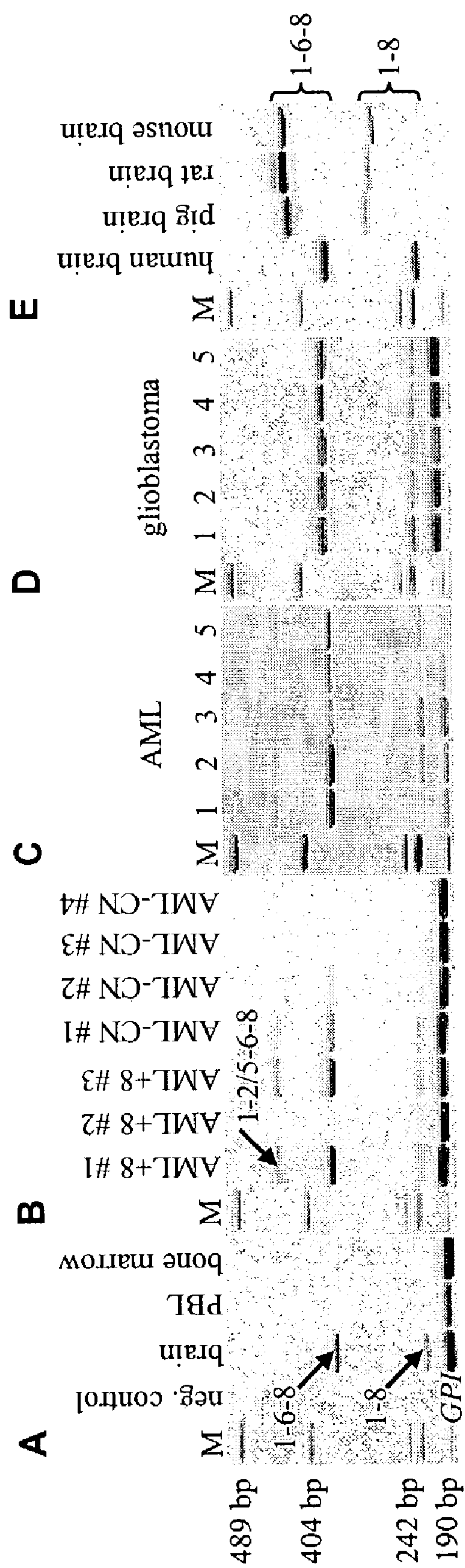


Fig. 3

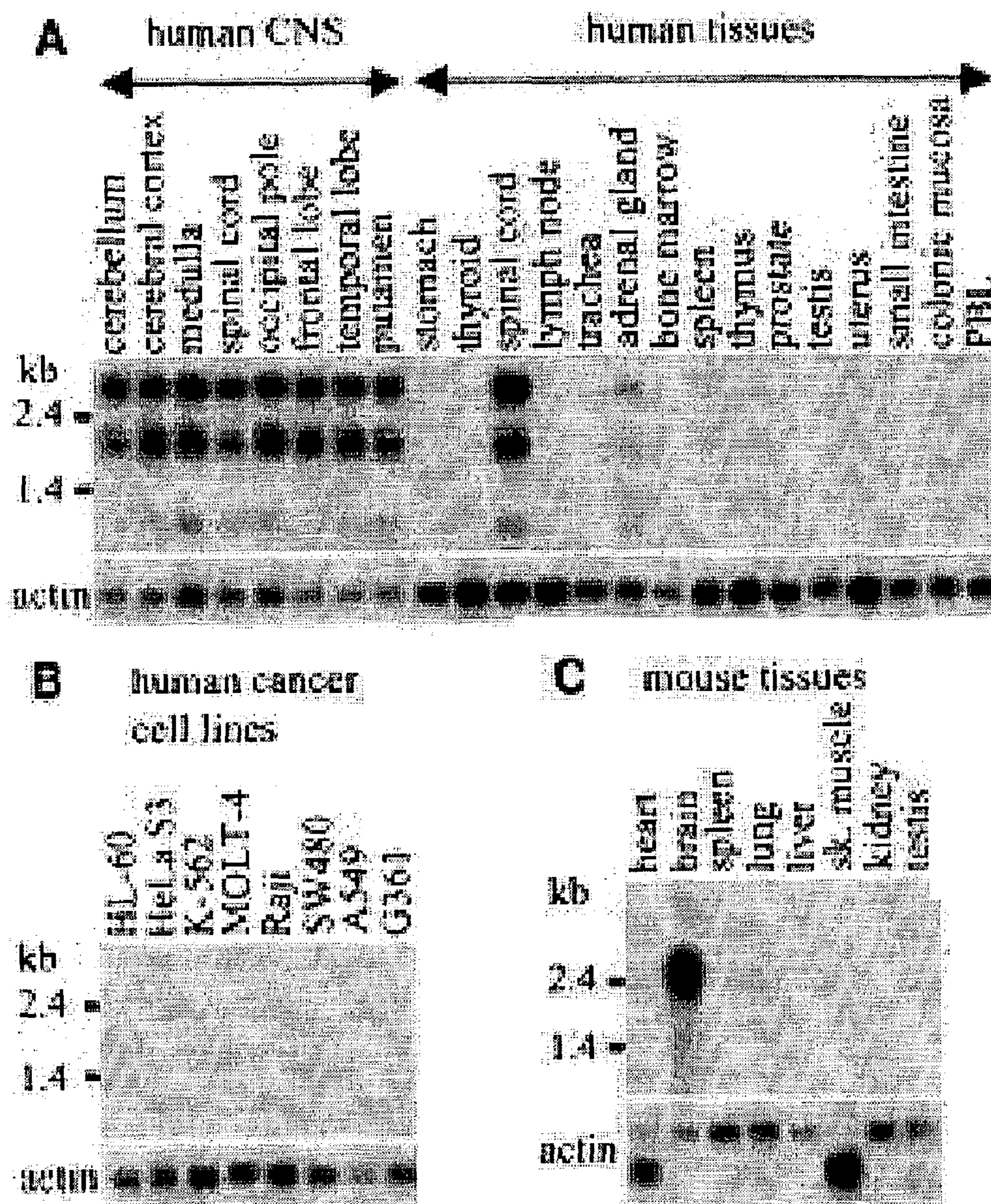


Fig. 4

1-6-8

gagagggcccgga cuaggggcggcgggcaccgcaggagcuccgcgcggcugcagcgcgggcgggagcggggacgc
gaugucgcgcgcgcgcgcgcuccuugcgggcggggcugcgcgcuccggggcugagccgcgcgcagagccgacagccg
agcagccgcuggggcgcucccgcggcgcaggaggAUGGGCUGCGGCGGGAGCCGGGCGGAUGCCAUCGAGCCCCGC
UACUACGAGAGCUGGACCCGGGAGACAGAAUCCACCUGGCUCACCUACACCGACUCGGACGCGCCGCCAGCGCC
GCCGCSCCGGACAGCGGCCCCGAAGCGGGCGGCCUGCACUCGGGCAUGCUGGAAGAUGGACUGCCCUCCAAUGGU
GUGCCCCGAUCUACAGCCCCAGGUGGAAUACCCAACCCAGAGAAGAAGACGAACUGUGAGACCCAGUGCCCAAU
CCCCAGAGCCUCAGCUCAGGCCCCUCUGACCCAGAAACAGAAUGGCCUUCAGACCACAGAGGCUAAAAGAGAUGCU
AAGAGAAUGCCUGCAAAAGAAGUCACCAUUAUGUAACAGAUAGCAUCCAACAGAUUGGACAGAAGUCGAAGAAUC
ACAAAGAACUGUGUCAACUAGcagagaguccaagcagaagggcagauggacuucucaguguccuucacgggcacu
ggauecccaucaagaaccuugaagaaguggcugcccccugcuggaccugaaucucacugagucccuggcaagaçu
gucuuaccuggcagcaaacugcugccugauuuuguugggaccuucugagccuucuaucuaucuauguaaauguaauug
gcacagugcuaucuauguuAAUAAAcugcaaaugugcaguucaguuuugucucuuugcaacuccuguaauacgggu
cugguguaaaaguagugaguuuaagcuaacaggucaguuuaugaaacagaaaaguaggaaugcauuuucuggguga
aagagucacaccuagugcuaauaacucuccugcccgaugauaguguaauucuguuucaggcaagcuuauucuuuccu
ucuuucauuuuuaauuugucuaauacaaucuuaccagguucacuuaaaagcuggcuuucacuccaacucuaaacc
cacauauugaaaaaaucaagguacaggaaaacuccuuguaucuccuuguuuccuagcuugguaugagacagauccg
gauccaguuucccaugcaccaccccacugcccgauggcaugucuuugggaggugucugugaagcagucacuaaccugc
uccucaucugccuggaaaguccuccuauuccaguguccaaguuggccuccaguccuuaaugucaccaugcuugug
gccaaugcauccaaauaaggauaccccucagggcucagcuaagacauugcaauuuugcauagcuuuccaguucccu
uugcuugucuuucugacugucuuucccucucuaucggggucacuugcaauuuuaaaucaaaagauugaacacugcgua
ggagaggggagaugauccagagacauugggcagcaggcauggcuucccccugggccucucuguaacacugcccagga
cugucauuuuggcaucugcaaaaggaaucacuuuagaaagccagcaccugguugauguguaauucacugacauua
gauugaugugcacugcauuagaaaugagguagcugacacagaaaaaggauuuuugauaggaaauuuuucuaugu
augucuuugaaacauguuacucuggaaguauuuuccuccaaaguuuagauagcauuuuucaaaggauuguaaca
ugccuggggaauugggaaagauaggacuaaaguugugccaaacuaauucAAUAAuuccauguuuagcagaaauagg
cagccuauuggguguauguuuauguaacauaguccagagaacugacaugcagggucaaaagucagauacgcaaccu
ccuuaucugcuaaacucuguuauucuucaaacacacacggggguagugucuuuuuccuuccuuccuuccauuggca
gauuguaauuuuauucacaaaacauuaaauuguccauccugugccagguacuaugcagauguugagggauuuuggg
ucuggguagucgugacuaucuaucuccugaaucaaacagugacuucauaacuaggagacugaauuagaccuuuagg
uauagugugugugcaaaucacucugcaauuggaaacuuuuauauucaggguaggguugugucuuuaacuaggugu
ucuaaucaauguacaagacuuuaccuacacgcaacuauaguuuuucuaaaccuucacuaauuuugugauucuuug
agaaagggcuuuuaggaacuuuauugucuaaaaaaauuuuuuuaacaauaauaagauaaaagaaaaaccugugauu
cauauugcccccacuggcauuacucagcaggagccccagcugccaaagguuggcagugauccugcaaguucaagg
gcucuuucucccugggggaugugcuuuguggcuucucuuuacagcuuuguuucugcaucaguucacugcugcaugu
uguuuggaauuuaucaaccuuaagaaagugucucuguuuuauauagaaacacuuucacuuacaggggagaagga
aaugcagggcacaugaucuggcccccagaaacauucuggauuuaccgagacagcaaccagaaguuaaaccuau
gugacuaaaaaaugcaucuggcuacuuuuuauuguauguaugagacagaaacuaaaccuuaucuaucuuuaggau
accacuuuucauugcaaaaguugugucAAUAAgucauuauuuuuaacau

Fig. 5

1-8

gagagggccccggacuaaggggcggcggggcacgcaggaagcuccgcgcggcugcagcgcgggcgggagcgggg
acgcgaugucgcgcgcgcgcgcuccuugcgggcggggcugcgcuccgcggggcugagccgcgcgcagagcc
gacagccgagcagccgcugggcgccuccgcggcgaggaaggAUGGGCUGCGGCGGGAGCCGGGCGGAUGCC
AUCGAGCCCCGCUACUACGAGAGCUGGACCCGGGAGACAGAAUCCACCUGGCUCACCUACACCGACUCGGA
CGCGCCGCCAGCGCCGCCGCSCCGGACAGCGGCCCCGAAGCGGGCGGCCUGCACUCGGGCUAAAAGAGAU
GCUAAGAGAAUGCCUGCAAAGAAGUCACCAUUAUGUAACAGAUAGCAUCCAACAGAUUGGACAGAAGUCC
AAGAAUCACAAAGAACUGUGUCAACUAGcagagaguccaagcagaagggcagauggacuucuuacagugucc
uucacgggcacuggaucccaucaaaagaaccuugaagaaguggcugcccccugcuggaccugaauucuaacuga
guccucuggcaagacugucuuaccuggcagcaaacugcugccugauuuguugggaccuucugagccuucua
uuaucauguaaauuguaauuggcacagugcuuacauauguuAAUAAAacugcaaaugugcaguucaguuuugcu
cuuugcaacuccuguaauacggucuggguguaaaaguagugaguuaaagcuacaggucaguuuaugaaacag
aaaaguaggaauugcauuuucugggugaaagagucacacccuugugcuauaacucuccugcccaugauagug
uaucuguuucaggcaagcuuauucuuuccuucuuuacauuuuaauuugucauuacaaaucuuaccaggu
ucacuuaaaagcuggcuuucacuccacucuaaaacccacauauugaaaaaucaagguaacaggaaaacuccu
uguuauccuuguuuccuagcuugguauagagacagauccggauccaguuucccaugcaccaacccacugccc
auggcaugucuuugggaggugucugugaagcagucuuaccugcuccuacucugccuggaaaguccuccuau
uccaguguccaanguuggccuccaguccuuaaugucaccaugcuuguggccaaugcauccaaauaaggauac
cccucagggcucagcuagacauugcaauuuugcauagcuuuccaguucccuuugcuugucuuucugacugu
cuucccucucuaucggggucacuugcaauuguaaucaaaagauugaacacugcguaaggagaggggagaugau
ccagagacauguggcagcaggaugggcuuccccuuggccucucuguacacugcccccaggacugucuuuug
gcaucugcaaaaggaaucauuuagaaagccagcaccugguugauguguaauucauacugacauuagauugau
gugcacugcauuagaaaugagguagcugacacagaaaaaggauuuuugauaggaauaauuuuucuaugau
ucuuagaaacauguucaucuggaaguuuuuccuccaaaguuuaggaugauuuuucaaggauuguaaac
augccuggggaauugggaaagauaggacuaaaguugugccaaacuauaucAAUAAAuuccauguuuagcagaa
auaggcagccuauuggguguuauguuuauguaacauaguccagagaacugacaugcagggucaaaagucagau
acgcaaccuccuuaucugcuaaacucuguuauucuucaaacacacacguggguagugucauuuuuccuuccu
ccuuccauugggcagauuuguaauuuuauucacaaaacauuaaauguccauccugugccagguacuaugcaga
uguugaggggaauuugggggucugguuagucgugacuaucuaucucugaaucaaacagugacuucuaaacuagga
gacugaauuagaccuuaagguauaguguguguuugcaaaucacucugcaauuggaaacuuaauuucaggg
uagguuugugucuuaaacuaggguuucuaaucaauguacaagacuuaaccuacacgcaacuauaguuuu
cuuaaccuucacauuuuugugauucuuugagaaagggcuuuaggaacuuauguuuuaaaaaauguuuu
aacaauaauaagauaaaagaaaaaccugugauucauauuguccccacuggcauuacucagcaggagccccc
gcugccaaagguuggcagugaucugcaaguucaagggcucuuucucccugggggaugugcuuuguggcuuc
ucuuuacagcuuuguuucugcaucaguucacugcugcauguuuguuuggaauuuuaucccuuaagaaagugu
cucuguuuuauauagaaacacuuucucacuuacagggggagaaggaaaugcagggcacauagaucuggccuc
cccagaacaauucuggauuucacggagacagcaaccagaaguuaaaccaugugacuuaaaaugcaucuggcu
acuuaauucauguauguaugagacagaaacuauuccuuacuauccuauuaggauaccacuuaucuuugcaaa
quuugugucAAUAAAgucauuuauuuuuaacau

Fig. 6

1-5-6-8

gagagggcccggaacuaggggcccggggcaccgcaggagcuccgcgcgggcugcagcgcggggcgggagcggggac
gcgaugucgcccgcgcgcgcuccuugcggggccggggcugcgcgcuccggggcugagccgcgcgcagagccgaca
gccgagcagccgcgcggggcgcuccccgcgggcgcaggaggAUGGGCUGCGGCGGGAGCCGGGCGGAUGCCAUCGAG
CCCCGCUACUACGAGAGCUGGACCCGGGAGACAGAAUCCACCUGGCUCACCUACACCGACUCGGACGCGCCGC
CCAGCGCCGCGCSCCGGACAGCGGCCCCGAAGCGGGCGGCCUGCACUCGGUUCUGGAGGCUGAGAAGUCCAA
GAUCAAGGCACCAACAGAUUCAGUGUCUGAUGAAGGCUUGUUCUCUGCUUCAAGAUGGCACCUCUUGCUGUG
UUCUCACAUGGCAUGCUGGAAGAUGGACUGCCCUCCAUGGUGUGCCCCGAUCUACAGCCCCAGGUGGAAUAC
CCAACCCAGAGAAGAAGACGAACUGUGAGACCCAGUGCCCAAUCCCCAGAGCCUCAGCUCAGGCCCUUGAC
CCAGAAACAGAAUGGCCUUCAGACCACAGAGGCUAAAAGAGAUGCUAAGAGAAUGCCUGCAAAAGAAGUCACC
AUUAAUGUAACAGAUAGCAUCCAACAGAUUGGACAGAAGUCGAAGAAUCACAAAGAACUGUGUCAACUAGcaga
gaguccaagcagaagggcagauggacuucuuucaguguccuucacggcacuggaucccaucaaaagaaccuugaa
gaaguggcugccccuugcuggaccugaauucuaucugagucccuggcaagacugucuuaccuggcagcaaacug
cugccugauuuuguugggaccuucugagccuucuaucuuaucauguaaauguaauuggcacagugcuuacauaugu
uAAUAAAcugcaaaugugcaguucaguuuugucucuuugcaacuccuguaauacggucugguguaaaaguagug
aguuaaagcuacaggucaguuuuagaaacagaaaaguaggaaugcauuuucugggugaaagagucacaccuua
gugcuauaacucuccugcccagauaguguaauucuguuucaggcaagcuuauucuuuccuucuuucauuuuuaa
auauugucuuuacaaucuuaccagguucacuuaaaagcuggcuuucacuaacacucuaaaccacauauugaa
aaaaucaagguacaggaaaacuccuuguuauccuuguuuccuuaagcuugguauagagacagauccggauccagu
ucccaugcaccaaccacugcccagggcaugucuuugggaggugucugugaagcagucacaccugcuccucau
cugccuggaaaguccuccuauuccaguguccauguuggccuccaguccuuaaugucaccaugcuuguggccaa
ugcauccaaauaaggauaccccucagggcucagcuagacauugcaauuuugcauagcuuuccaguucccuug
cuugucuuucugacugucuuuccucucuaucggggucacuuugcaauuguaaucaaaagauugaacacugcgua
ggagaggggagaugauccagagacauugggcagcaggcauggcuuccccuuggccucucuguaacacugccccag
gacugucuuuuuggcaucugcaaaaggaaucauuuagaaagccagcaccugguugauguguaaucauacugac
auuagauugaugugcagucuuuagaaugagguagcugacacagaaaaaggauguuuugauaggaauaauuu
ucuaguaugucuuugaacauguucaucuggaaguauuuuccuccaaaguaauguagcauuuuucaaaggau
uguuaacaugccuggggaugggaaagauaggacuaaaguugugccaaacuaauucAAUAAauccauguuuag
cagaaauaggcagccuauuggguguuauuguuuuaguaacauaguccagagaacugacauugcaggucaaaaguc
gauacgcaaccuccuuaucugcuaacucuguuauucuucaaacacacacguggguagugucuuuuuccuuccu
uccuuccauuggcagauuguaauuuuauucacaaaacauuaaauguccauccugugccagguacuaugcagau
guugaggggaauuuggggucugguagucgugacuaucuaucuccugaauucuaacagugacuucuaaacuaggagac
ugaauuagacccuuaagguauagugugugugcaaaucacucugcaauggaaacuuuuauauucaggguaggu
uugugucuuaaacuagguguuuaucaauuaguaacaagacuuuaccuacacgcaacuauaguuuuuucaaacc
uucaucauuuugugauucuuugagaaagggcuuuuaggaacuuuauuguuuaaaaaauguuuuuaacaaaua
agauaaaagaaaaaccugugauucuauguccccacuggcauuacucagcaggagccccagcugccaaaggu
uggcagugauccugcaaguucaagggcucuuucucccuggggauugugcuuuguggcuucucuuuacagcuuug
uuucugcaucaguucacugcugcauguuuggaauuuuacaccuuaagaaagugucucuguuuuauauaga
aacacuuucacacuuacaggggagaaggaaugcagggcacauagucuggccucccccagaacaucuggauu
ucacgggagacagcaaccagaaguuaaaccuugugacuaaaaaugcaucuggcuacuuuuuauuguaugua
gacagaaacuaauccuuaucuaucuuuaggauaccacuuuucuuugcaaaaguugugucAAUAAAgucuuu
auuuuaaaca

Fig. 7

[illegible]

Fig. 8

1-5-6-7-8

[illegible]

Fig. 9

1-2-6-8

[illegible]

Fig. 10

1-2-5-6-8

gagagggcccgga cuagggggcgggcgggcaccgcaggagcuccgcggcgggcugcagcgcgggcggggagcggggaacgcga
ugucgcgcgcgcgcgcuccuugcgggcgggcgggcugcgcgcuccggggcgugagccgcgcgcagagccgacagcccgagca
gccgcugggcgccuccgcggcgcgaggaggAUGGGCUGCGGGCGGAGCCGGGCGGAUGCCAUCGAGCCCCGCUACUAC
GAGAGCUGGACCCGGGAGACAGAAUCCACCUGGCUCACCUACACCGACUCGGACGCGCCGCCAGCGCCGCCGCCSCC
GGACAGCGGCCCCGAAGCGGGCGGCCUGCACUCGGCCCAUUACCCUCUUGCCUUGCACUUGCCUGGAGAGACAACA
GUUUAGGGGCUUCUGCUGGUUCAAGAAGGACUGUGCAGGUAGCAUGGCCACACACCAUGUACAGUUCUGGAGGCUGAG
AAGUCCAAGAUCAAGGCACCAACAGAUUCAGUGUCUGAUGAAGGCUUGUUCUCUGCUUCAAGAUGGCACCUCUUGC
UGUGUUCUCACAUGGCAUGCUGGAAGAUGGACUGCCCUCCAAUGGUGUGCCCCGAUCUACAGCCCCAGGUGGAUAC
CCAACCCAGAGAAGAAGACGAACUGUGAGACCCAGUGCCCAAUCCCCAGAGCCUCAGCUCAGGCCCUUGACCCAG
AAACAGAAUGGCCUUCAGACCACAGAGGCUAAAAGAGAUGCUAAGAGAAUGCCUGCAAAAGAAGUCACCAUUAUGU
AACAGAUAGCAUCCAACAGAUUGGACAGAAGUCGAAGAAUCACAAAGAACUGUGUCAACUAGcagagaguccaagcag
aagggcagauggacuucucaguguccuucacggcacuggaucuccaucaagaaccuugaagaaguggcgccccuu
gcuggaccugaauucuaucugagucccuggcaagacugucuuaccuggcagcaaacugcugccugauuuuguuggacc
uucugagccuucuaucuaucauguaaauguaauuggcacagugcuuacauauguuAAUAAAcugcaaaugugcaguuc
aguugugucuuugcaacuccuguaauacggucugguuuaaaguagugaguuaaagcuacaggucaguuaugaaa
cagaaaaguaggaaugcauuuucugggugaaagagucacaccuuagugcuuaaacucuccugcccagauagugua
ucuguuucagggaagcuuauucuuuccuucuuucaaauuaaauugucuuuacaaucuuaccagguucacuuaaa
agcugggcuuucauuccaacucuaaaacccacauauugaaaaaaucaagguacaggaaaacuccuuguaucuccuuguuc
cuuagcuugguauagagacagauccggaucaguuucccaugcaccacacacugcccagggcaugucuuugggaggug
ucugugaagcagucuuaccugcuccuucacugccuggaaagucuccuauuccaguguccauguuggccuccagucc
uuauugucaccaugcuuguggccaaugcauccaaauaaggauacccucagggcucagcuagacauugcauuuugc
auagcuuuccaguuuccuugcuugucuuucugacugucuuuccucucuaueggggucacuuugcaauuguaaaucaa
agauugaacacugcguaaggagaggagauagauccagagacauugggcagcaggcauggcuuccccuuggccucucug
uacacugccccaggacugucuuuuggcaucugcaaaaggaaucacuuuagaaagccagcaccugguugaugugua
cauacugacauuagauugaugugcagucuuuagaaugagguagcugacacagaaaaaggauuuuugauaggaa
aaauuucuaugauugucuuuagaaacauugucacuggaaguauuuuccuccaaaguaauguagcauauuuucaagga
uuguaaacaugccugggauugggaaagauaggacuaaaguugugccaaacuauaucAAUAAuuccauguuuagcag
aaauaggcagccuauugguguuauuguuuaguaacauaguccagagaacugacaugcaggucaaaagucagauacgc
aaccuccuuaucugcuaacucuguuauucuucaaacacacacgugggguagugucuuuuuccuuccuuccuuccauug
gcagauuguaauuuuauucacaaaacauuaaauuguccauccugugccagguacuaugcagaugaugggauuuuggg
gucugguuagucgugacuaucuaucuccugaauucuaacagugacuucauaacuaggagacugaauuagaccuuuaggu
auaguguguguuugcaauacacucugcaauaggaaacuuuuauauucaggguagguuugugucuuaaacuagguguc
aaucaauguaacaagacuuuaccuacacgcaacuauaguuuuucuaaacuccuacauuuugugauucuuugagaaa
gggcuuuuaggaacuuuauugucuaaaaaauguuuuuuaacaauaauaagauaaaagaaaaaccugugauucuuu
ccccacuggcauuacucagcaggagccccagcugccaaaggguuggcagugauccugcaaguucaagggcucuuuc
cccugggggaugugcuuuguggcuucucuuuacagcuuuguuucugcaucaguuacucugcugcauguuguuuggaau
uauacccuuaagaaagugucucuguuuuauauagaaacacuuucacuuacaggggagaaggaaugcagggcaca
ugaucuggcccccagaaacauucuggauuucacggagacagcaaccagaaguuaaaccuugugacuaaaaaugca
ucuggcuacuuuuuauuguauguaugagacagaaacuaauccuuaucuaucuaauaggauaccacuuuucuuugcaa
aguugugucAAUAAAgucuuuauuuuuaacau

Fig. 11

1-2-3-6-8

[illegible]

Fig. 12

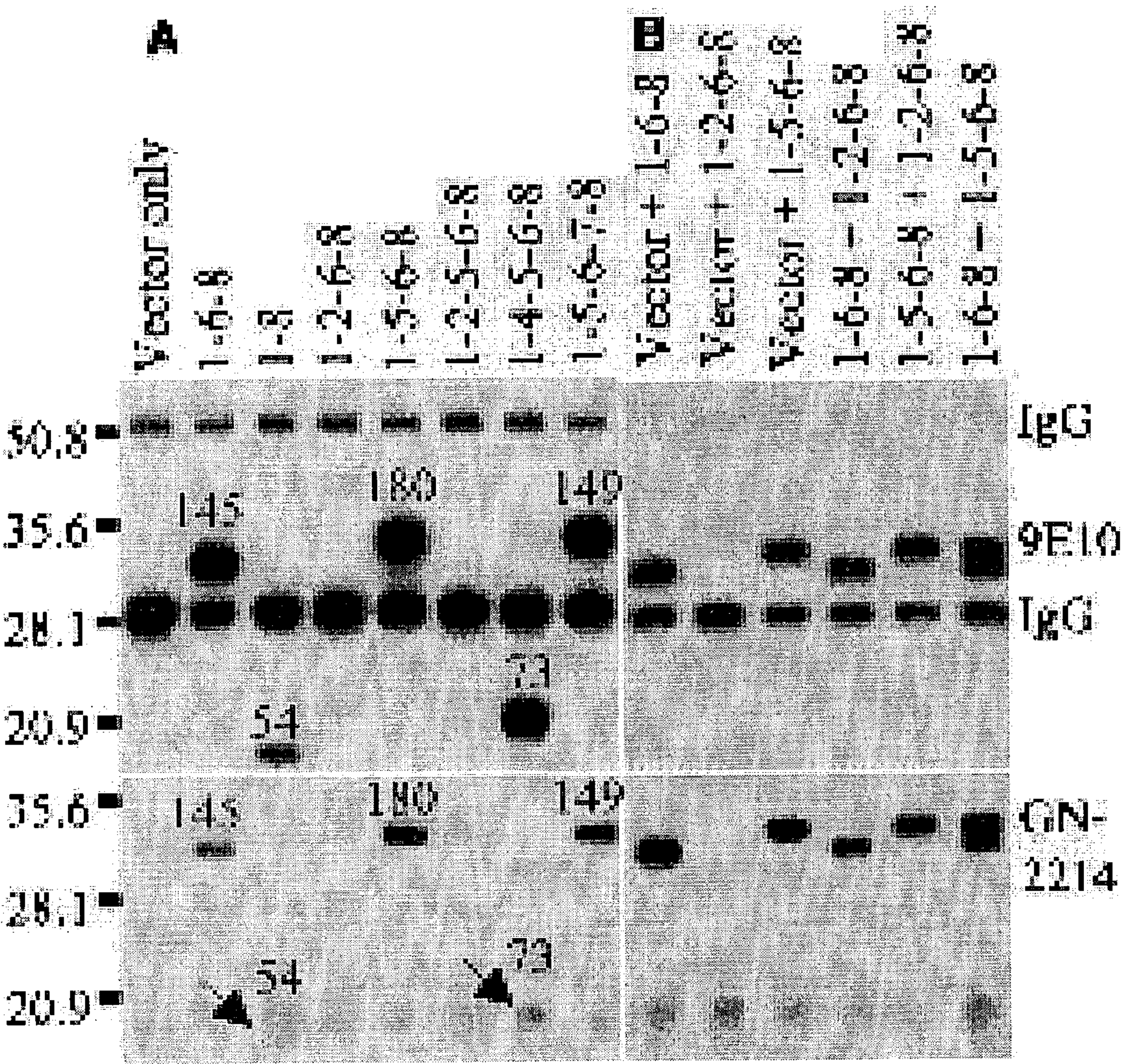


Fig. 13

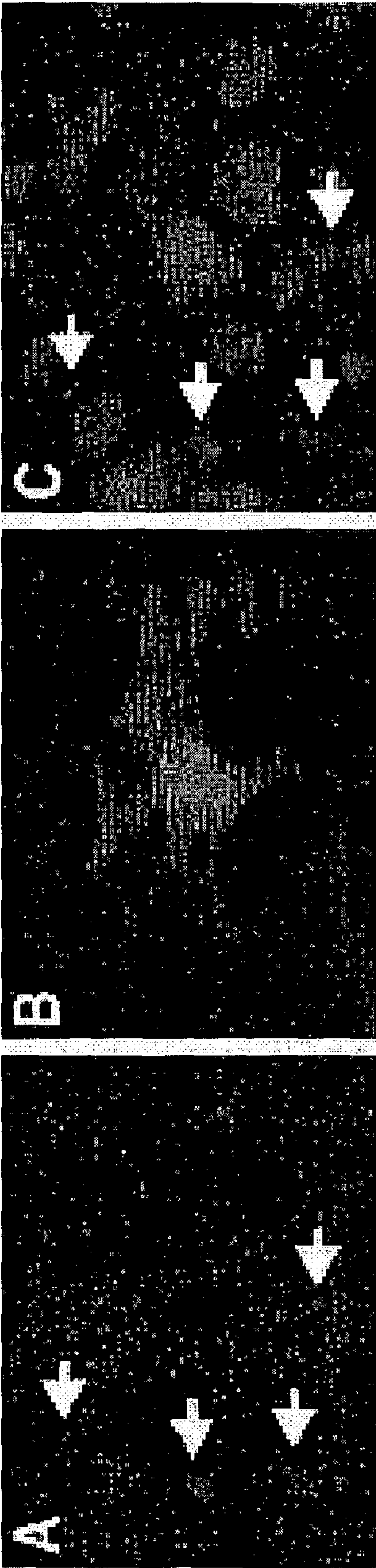


Fig. 14

1-6-8

MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
GMLEDGLPSNGVPRSTAPGGIPNPEKKTNCETQCPNPQSLSSGPLTQKQNGLQ
TTEAKRDAKRMPAKEVTINVTD SIQQMDRSRRITKNCVN

1-8

MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
G

1-5-6-8

MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
VLEAEKSKIKAPTDSVSDEGLFSASKMAPLAVFSHG MLEDGLPSNGVPRSTAP
GGIPNPEKKTNCETQCPNPQSLSSGPLTQKQNGLQTTEAKRDAKRMPAKEVTI
NVTD SIQQMDRSRRITKNCVN

1-4-5-6-8

MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
GCLEEHYHLTALQKVGHPNH

1-5-6-7-8

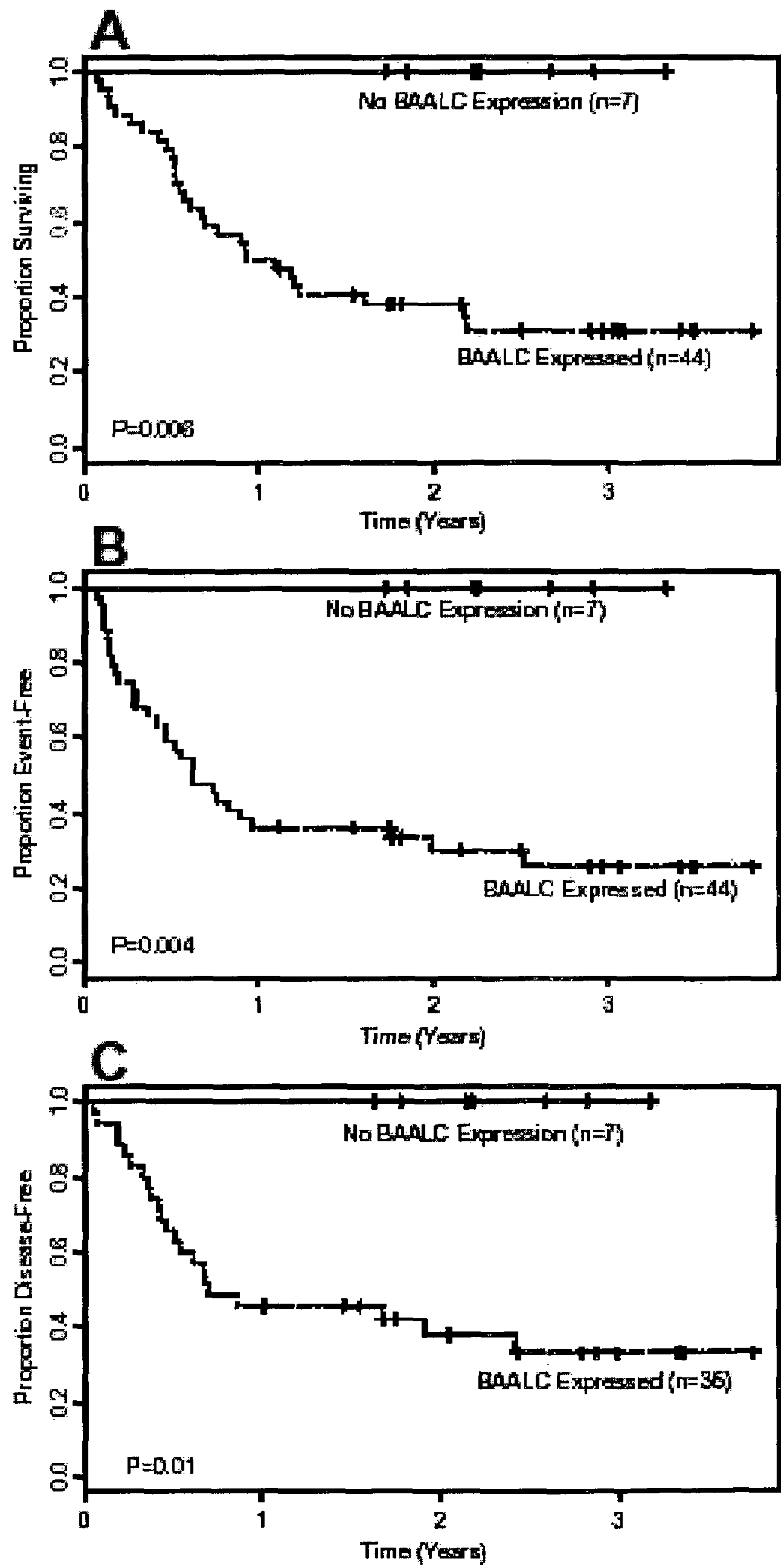
MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
VLEAEKSKIKAPTDSVSDEGLFSASKMAPLAVFSHG MLEDGLPSNGVPRSTAP
GGIPNPEKKTNCETQCPNPQSLSSGPLTQKQNGLQTTEVLLPS

1-2-6-8, 1-2-5-6-8 and 1-2-3-6-8

MGCGGSRADAIEPRYYESWTRETESTWLTYTDS DAPPSAAAPDSGPEAGGLHS
AHYPLAFALAWRDN SLGALLVQEGLCR

Fig. 15

Fig. 16



BAALC EXPRESSION AS A DIAGNOSTIC MARKER FOR ACUTE LEUKEMIA

This application claims priority to U.S. Provisional Application Ser. No. 60/348,210, filed Nov. 9, 2001, which is incorporated herein in its entirety.

This invention was made, at least in part, with government support under National Institutes of Health Grant No. 5P30CA016058. The U.S. government has certain rights in the invention.

BACKGROUND

Leukemias comprise approximately 2% of adult cancers and are a heterogeneous group. There are two broad categories of leukemias. Acute leukemias arise when there is a block in the normal differentiation of cells to mature blood cells that results in large accumulations of immature cells or blasts in the blood. Examples of such cancers are acute myelogenous leukemia (AML; other names are acute myeloid leukemia and acute nonlymphocytic leukemia) and acute lymphoblastic leukemia (ALL). In chronic leukemia, on the other hand, there is unregulated proliferation of cells that have differentiated to mature blood cells. Examples of such cancers are chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML). CML has a chronic phase which then progresses to a phase called blast crisis where immature, blast cells are present in the blood. Both acute and chronic leukemias involve the myeloid cells of the bone marrow, including white cells, red cells, megakaryocytes and cells of the lymphoid lineage.

The cytogenetics of many leukemias are characterized by balanced chromosomal translocations that give rise to gene rearrangements. In acute myeloid leukemia (AML) for example, about 55% of adult de novo cases have clonal cytogenetic abnormalities, many of which are specific translocations. However, in the remaining cases, no visible cytogenetic abnormalities are found, although genetic changes are detected methods other than cytogenetics. In adult acute lymphoblastic leukemia (ALL), the proportion of patients with no cytogenetic abnormality is about 31%.

Tumors of the central nervous system (CNS) comprise primary brain tumors, primary intraspinal tumors, and tumors that metastasize to the CNS. Brain tumors comprise astrocytomas, glioblastomas, medulloblastomas, and others. An extracranial pediatric tumor, neuroblastoma, arises in pluripotent neural crest cells of the sympathetic nervous system.

Prostate cancer is an epithelial cell cancer of men. Most are adenocarcinomas. Tumorigenesis progresses from normal to hyperplastic prostate to well and poorly differentiated carcinoma.

Many cancers, including leukemias, CNS and prostate cancers suffer from the problem of late detection in patients. Also, even when such cancers are detected in a patient, it is often difficult to predict lifespan of, or to determine the optimal therapy for, the particular cancer in the particular patient. However, cancers are genetic diseases that are associated with changes in cellular DNA (i.e., genetic changes). Because occurrence of such DNA changes precedes the appearance of phenotypic changes characteristic of cancer cells, it is advantageous to use detection of such early genetic changes as an aid to cancer diagnosis. Also, because a single cancer type, as identified phenotypically or pathologically, may include cancers that can be subgrouped based on classification of genetic changes therein, detection of these genetic changes may provide improved patient prognosis and selection of more efficacious therapy, based on the subgroupings.

Although the specific genetic changes associated with some cancers are known, in other cancers the associated genetic changes are not known. Even if the genetic changes are not known, it may be possible to identify additional molecular changes resulting from the genetic changes contributing to cancer. For example, a genetic change in a cancer cell may result in changes in gene expression (i.e., transcription and/or translation) of multiple genes in a cancer cell. A gene which is not normally expressed in a particular cell type may come to be expressed in cancer cells, or a gene that is expressed at low levels in normal cells may come to be expressed at high levels in cancer cells. Such gene expression changes may be diagnostically and prognostically useful alone or may be used together with already identified cancer-associated genetic and gene expression changes in multivariate analysis for purposes of prognosis and selection of effective anticancer therapy.

Therefore, it would be advantageous to identify and characterize genetic changes and gene expression changes present in cancer cells, particularly in leukemias, CNS and prostate cancers, that can be used to more effectively diagnose a specific cancer, predict its outcome in a patient, and aid in selecting an efficacious therapy.

SUMMARY OF THE INVENTION

A new gene, BAALC (Brain and Acute Leukemia, Cytoplasmic) has been identified that has eight exons, and expresses eight alternatively-spliced transcripts and six protein isoforms. The BAALC gene, while normally expressed in central nervous system (CNS) tissues, adrenal gland, thyroid and spleen, is overexpressed in subsets of leukemias, in certain CNS cancers, and in prostate cancer.

Thus, the present invention provides oligonucleotides and polynucleotides sequences identical or complementary to sequences within BAALC exons or transcripts. The oligonucleotides are used as primers and probes for detecting BAALC expression and overexpression in a biological sample obtained from a patient.

The present invention also provides polyclonal and monoclonal antibodies that specifically bind to one or more BAALC protein isoforms, as well as peptide immunogens for preparing the antibodies. The antibodies are used for detecting expression and overexpression of BAALC proteins or polypeptides in a biological sample obtained from a patient.

The present invention also provides methods of characterizing certain leukemias in a patient. One such leukemia is acute myelogenous leukemia (AML). The method comprises assaying a biological sample obtained from a patient for the presence of one or more BAALC transcripts or protein isoforms, wherein overexpressed levels of BAALC transcripts or protein isoforms indicate an aggressive form of AML. Another such leukemia is chronic myelogenous leukemia (CML). This method comprises assaying a biological sample obtained from a patient for the presence of overexpressed levels of BAALC transcripts or protein isoforms wherein overexpressed levels in the CML cells indicates the cells have entered the stage of blast crisis.

The present invention also provides methods of diagnosing and characterizing prostate tumors. The method comprises assaying a biological sample obtained from a patient for the presence of overexpressed levels of BAALC transcripts or protein.

The present invention also provides a kit for characterizing AML, CML or prostate cancer wherein the kit comprises primers containing sequences identical or complementary to sequences within specific BAALC exons. The kit may also

comprise a probe containing a sequence complementary to a sequence contained within a polymerase chain reaction (PCR) product obtained using the primers.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the genomic organization and transcripts of the human BAALC gene. (A) BAALC is located between ATP6C proximal and FZD6 distal in human chromosome 8q22.3 and covers 90 kb of genomic sequence. The region in the three BAC clones shown were assembled as shown. BAALC has eight exons, depicted by boxes, with exon 8 containing three polyadenylation signals (triangles) in the 3' untranslated region (UTR) leading to three differently sized transcripts (FIG. 4A). Exon 1 contains the ATG start codon and a 5' UTR (diagonally striped). (B) The two transcripts 1-6-8 and 1-8 are detected in neuroectoderm tissues, and six more transcripts produced by alternative splicing are detected mainly in leukemias. The extent of the resulting coding regions are horizontally striped, and the protein sizes are indicated above.

FIG. 2 shows the DNA sequences of the eight exons of the human gene. Exons 1, 2, 3, 4, 5, 6, 7 and 8 are designated as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8, respectively. Lower case indicates untranslated regions. Upper case indicates translated regions. AATAAA sequences indicated by upper case letters indicates putative polyadenylation sites.

FIG. 3 shows comparative reverse transcription polymerase chain reaction (RT-PCR) results using primers in exons 1 and 8 of human BAALC in biological samples from brain, peripheral blood leukocytes (PBL), bone marrow (BM), 12 cases with AML, and 5 glioblastoma tumors with GPI serving as an internal control. M, size marker. (A) Exon 6 was alternatively spliced in brain and leads to transcripts 1-6-8 and 1-8. Transcript 1-6-8 was more highly expressed than transcript 1-8 in brain. No BAALC expression was detected in PBL, but faint expression occurred in BM. (B) The three cases of AML with +8 (trisomy for chromosome 8) and four cases of AML with normal karyotype (AML-CN) used in the cDNA-RDA experiment were studied individually by RT-PCR. The samples with AML+8, nos. 1 and 3, had very high levels of transcript 1-6-8, whereas the samples with AML-CN, nos. 1 and 2, had low levels of the transcripts. Moreover, two alternative transcripts, 1-2-6-8 and 1-5-6-8, were observed in the AML samples that were absent in brain. (C) Samples from five cases containing AML had alternative transcripts when BAALC was overexpressed. (D) Although the five glioblastoma tumor samples had transcripts 1-6-8 and 1-8, which were highly expressed, they were distinguished by their lack of expression of the alternative transcripts. (E) Conservation of BAALC splicing of transcripts 1-6-8 and 1-8 in brain samples from four mammalian species shown by RT-PCR with primers in exons 1 and 8.

FIG. 4 shows the tissue-expression pattern of BAALC. (A) Northern blots probed with human transcript 1-6-8. High expression of BAALC was restricted to neural tissues, and low expression was seen in the neuroectoderm-derived tissues adrenal gland, thyroid, and spleen. Note undetectable levels of BAALC in samples from BM, PBL, and lymph nodes, as well as in eight human cancer cell line samples (B). BAALC was expressed as three differently sized transcripts of about 1, 2, and 3 kb, because of the alternative usage of the three poly(A) signals in exon 8. (C) A mouse Northern blot probed with BAALC transcript 1-6-8 from mouse displayed a major 2.7-kb transcript exclusively in brain, indicating the

same neuroectoderm-specific expression as in human, with a clear preference for the second poly(A) signal.

FIGS. 5-12 show RNA sequences of BAALC alternatively spliced transcripts 1-6-8 (SEQ ID NO:9), 1-8 (SEQ ID NO:10), 1-5-6-8 (SEQ ID NO:11), 1-4-5-6-8 (SEQ ID NO:12), 1-5-6-7-8 (SEQ ID NO:13), 1-2-6-8 (SEQ ID NO:14), 1-2-5-6-8 (SEQ ID NO:15), and 1-2-3-6-8 (SEQ ID NO:16), respectively. These are the sequences of the transcripts shown schematically in FIG. 1B. In the sequences, lower case indicates untranslated regions. Continuous stretches of upper case letters indicates translated regions. After the translated regions, upper case AAUAAA indicates putative polyadenylation sites. Bolded letters indicate the beginning of an exon sequence (see FIG. 2).

FIG. 13 shows expression of BAALC protein isoforms. (A) Immunoprecipitation-Western blot analysis of isoforms 1-6-8 and 1-8, and the five alternative isoforms 1-2-6-8, 1-5-6-8, 1-2-5-6-8, 1-4-5-6-8, and 1-5-6-7-8 cloned into pcDNA3-5xMyc and transfected into 293 cells. BAALC protein detection was with anti-Myc mouse antibody 9E10 against the N-terminal Myc tag (Upper) or with anti-BAALC antibody GN2214, which is specific for amino acids encoded by exon 1 (Lower). The neuroectodermal transcripts, 1-6-8 and 1-8, produced the expected protein isoforms of 145- and 54-amino acids, respectively. Also as expected, transcripts 1-5-6-8, 1-4-5-6-8 and 1-5-6-7-8 produced the protein isoforms of 180-, 73- and 149-amino acids, respectively. Arrows indicate the small 54- and 73-amino acid isoforms. All isoforms contain an additional 63 amino acids from the N-terminal Myc tag. The secondary anti-mouse IgG antibody detected the IgG heavy and light chains from 9E10 (used for immunoprecipitation) on the Upper blot, which are not seen with the secondary anti-rabbit IgG antibody used on the Lower blot. (B) Coexpression in 293 cells of neuroectodermal isoform 1-6-8, stable isoform 1-5-6-8, and unstable isoform 1-2-6-8 with vector and with one another as indicated above the lanes. The last lane depicts coexpression of both stable isoforms. The results are confirmed with both 9E10 (Upper) and GN2214 (Lower).

FIG. 14 shows subcellular localization of BAALC protein. (A) Subcellular localization of human BAALC is shown after transfection of Myc-tagged isoform 1-6-8 into NIH/3T3 cells and staining with 9E10 and rhodamine-conjugated secondary antibody. (B) pcDNA3-green fluorescent protein served as a transfection control. (C) Triple-filter image. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). BAALC occurred as a few large inclusions (arrows) in the peripheral parts of the cytoplasm.

FIG. 15 shows amino acid sequences of the BAALC protein isoforms encoded by the RNA transcripts shown in FIG. 1B and FIGS. 5-12. BAALC proteins 1-6-8 (SEQ ID NO:17), 1-8 (SEQ ID NO:18), 1-5-6-8 (SEQ ID NO:19), 1-4-5-6-8 (SEQ ID NO:20), 1-5-6-7-8 (SEQ ID NO:21), as well as the same protein (SEQ ID NO:22) encoded by transcripts 1-2-6-8, 1-2-5-6-8 and 1-2-3-6-8 are shown.

FIG. 16 shows the clinical outcome of adult de novo AML patients below 60 years of age from whom PB samples with more than 50% peripheral blasts, and centrally reviewed clinical data were available. Biological samples from 7 out of 51 patients (14%) either did not contain detectable BAALC transcripts or contained normal (i.e., not overexpressed) levels of BAALC transcripts. Biological samples from 44 out of 51 patients (86%) contained elevated (i.e., overexpressed) levels of BAALC transcripts, defined as having higher BAALC expression levels than found in any of 10 PB samples from normal individuals (i.e., patients not having leukemia). Panels A, B and C are Kaplan-Meier curves. The log-rank test

was performed to determine whether there was a significant difference between the survival curves. (A) The median overall survival was 1.0 year (38% alive at 2 years) among the 44 positive patients compared with all 7 negative patients (100%) being alive at 2 years (censored for the outcome; $P=0.006$). (B) The median event-free survival for the 44 positive cases was 0.6 years (30% event-free at 2 years) compared with all 7 negative patients (100%) being event-free at 2 years (censored for the outcome; $P=0.004$). (C) The median disease-free survival for 35 positive cases (9 patients did not achieve complete remission) was 0.7 years (38% disease-free at 2 years) compared with all 7 negative patients (100%) being disease-free at 2 years (censored for the outcome; $P=0.01$).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Herein, “biological sample” means a sample of cells from a patient. These cells may be part of a tissue or organ sample obtained, for example, by biopsy, or they may be individual cells, for example, bone marrow cells, blood cells or even cells grown in culture. Preferably, such cells are cells obtained from peripheral blood. The cells from the blood that are assayed can be any or all cells present in the blood. Preferably, the cells assayed are blood-forming cells. More preferably, the cells are leukocytes. Such cells are commonly obtained by drawing a blood sample from a patient and then using standard techniques to purify or partially purify the cells from the blood. For example, a cellular fraction can be prepared as a “buffy coat” (i.e., leukocyte-enriched blood portion) by centrifuging whole blood for a short time at low speed (e.g., 10 min at 800 times gravity) at room temperature. Red blood cells sediment most rapidly and are present as the bottom-most fraction in the centrifuge tube. The buffy coat, containing the leukocytes, is present as a thin creamy white colored layer on top of the red blood cells. The plasma portion of the blood forms a layer above the buffy coat. Fractions from blood can also be isolated in a variety of other ways. One method is by taking a fraction or fractions from a gradient used in centrifugation to enrich for a specific size or density of cells.

The biological samples may be of normal cells, or may be of tumor cells, the tumor cells being benign or malignant. Generally, an assay that uses cells from such biological samples, herein, will be used to determine the presence of BAALC transcripts or proteins, or levels of BAALC transcripts or proteins. When such an assay is performed, the “test sample” will generally be a sample for which the presence or level of BAALC is unknown and is being tested to provide, for example, an indication of the aggressiveness of the cells. In such an assay, a “control sample” will preferably also be used. The control sample can be from normal (i.e., non-tumorigenic or non-neoplastic) tissue from the same patient from which the test sample is taken or can be from another person known or thought not to have the tumor that is present or thought to be present in the patient from whom the test sample is taken. Preferably, the control sample comprises the same type of cells that comprise the test sample. For example, if the test sample comprises leukocytes, it is preferable that the control sample is also a leukocyte sample.

The biological samples can be obtained from patients at various times. For example, a sample may be obtained from an individual who is suspected of having a tumor or cancer. Assay of such sample using the methods described below can indicate whether the individual has a tumor or cancer or can

provide characterization of the tumor or cancer cells. Samples may also be obtained from an individual known to have a tumor or cancer (i.e., the sample is taken after the patient has already been diagnosed). Assay of such sample using the described methods may have prognostic value to the individual. Multiple samples can also be taken from the same individual, for example, at different times after diagnosis. Assay of such samples can indicate whether the cancer or tumor is growing or spreading. Such assays on multiple samples are especially informative in the case where it is desired to determine the effect of a chemotherapeutic or other therapeutic agent or agents on the growth and progression of the tumor or cancer in the individual.

The test samples are preferably obtained from patients who are known to have or suspected of having a tumor or cancer. Methods for diagnosis of particular tumors or cancers in patients are well known in the art of medicine, oncology and hematology.

Herein, “assaying,” when used in reference to biological samples, preferably the cells in biological samples, refers to assessment or measurement of the presence and/or levels or concentrations of BAALC gene expression (transcripts or protein isoforms) in the samples. This assessment is done by detecting and/or measuring the levels of RNA transcribed from the BAALC gene or proteins which are translated from the RNA transcripts. As will be described subsequently, multiple transcripts, called alternative transcripts in the art, may be present when the BAALC gene is transcribed. Such alternative transcripts come from different combinations of exons encoded by the BAALC gene (see FIG. 2). Some or all of these transcripts are translated to produced BAALC proteins. The BAALC proteins that are obtained from translation of different alternative transcripts, may be somewhat different (in size and/or sequence) from one another depending on the combination of exons existing in the particular alternative transcript or on how the exon sequences in the transcripts are translated. Such different proteins are known in the art as protein “isoforms.”

Assaying these samples, with respect to BAALC expression, may involve detection or quantification of one or more specific alternative transcripts or protein isoforms. For example, one may be interested in determining the presence, level or concentration of one specific alternative transcript or protein isoform. Alternatively, assaying the samples may involve detection or levels of BAALC transcripts or proteins as a whole. For example, in determining the level or concentration of BAALC transcripts, the sum of the levels of all of the different alternative transcripts or protein isoforms may be used.

With regard to elevated levels or elevated concentrations of one or more BAALC alternative transcripts or protein isoforms, “elevated” means an increase in the amount of the transcript or isoform in the test sample as compared to the control sample. “Elevated in the test sample as compared to the control sample” describes a situation where the presence of BAALC transcripts or proteins is detected in the test sample and the amount, level or concentration of the BAALC transcripts or proteins in the test sample is greater than in the control sample. This means, in the control sample, that BAALC transcripts or proteins are either not detected, or that BAALC transcripts or proteins are detected, but are not present in amounts, levels or concentrations as high as are present in the test sample.

Therefore, to ascertain whether the test sample contains “overexpressed” levels of BAALC, a comparison of the levels in the test sample to the levels in one or more control samples is performed. Levels in a control sample or samples can be

represented by a single value or range of values. Preferably, an average of the BAALC levels in more than one control sample is used for comparison with the BAALC levels in the test sample. More preferably, an average of the BAALC levels from a number of control samples sufficient to provide a statistically significant comparison with BAALC levels present in the test sample is used. The control sample levels of BAALC may be determined at the same time at which BAALC levels in the test sample is determined. The BAALC levels in the control samples may also be predetermined, meaning that the levels have been determined before the time at which BAALC levels in the test samples are determined. In the case where BAALC levels in control samples are predetermined, the values are preferably normalized or standardized such that they can be legitimately compared with values for BAALC levels in test samples that are determined later.

With reference to overexpressed, increased or elevated levels of BAALC transcripts or proteins in the test sample, the amount of the increase can be of various magnitudes. The increase may be relatively large. For example, a large increase could be a 100% or more increase in BAALC expression in the test sample as compared to the control sample. However, the increase may be relatively small. For example, the increase may be less than 100%, less than 50%, or even less than a 10% increase of the transcript or protein in the test sample as compared to the control sample. Preferably, whatever the degree or magnitude of the increase, such increase is statistically significant. Methods for determining whether an increase is statistically significant are well known in the art of statistics and probability.

Comparison of the test sample to the control sample for the presence and/or levels of BAALC expression is used to characterize the tumor or cancer, i.e., to determine the "aggressiveness" of the tumor or cancer. A level of BAALC transcripts or proteins in the test sample that is higher than the level in the control sample indicates presence of an aggressive tumor or cancer. The extent or degree of the increase between the level of BAALC transcripts or proteins in the test sample and the control sample correlates with degree of aggressiveness of the tumor or cancer. Aggressiveness refers to the nature of tumor cell growth a patient. For example, an aggressive cancer has a higher probability of producing an unfavorable outcome in a patient than a cancer that is less aggressive. "Unfavorable outcome" normally refers to the probability that a patient will have a relatively short lifespan due to the aggressive nature of the cancer. Patients with a less aggressive cancer or cancer that is not aggressive are expected to have a longer lifespan than a patient with an aggressive form of the cancer.

In addition to predicting outcome in a patient, determination of BAALC overexpression, and tumor or cancer aggressiveness, is used for selecting an appropriate therapy for the patient with the tumor or cancer. In addition, determination of BAALC overexpression is used for determining if such a therapy is used and when it should be used to treat the patient.

Cloning of BAALC

The present invention provides a new gene, BAALC (Brain and Acute Leukemia, Cytoplasmic), expression of which is diagnostic for certain cancers and prognostic for patients with certain cancers. To clone the gene, a cDNA-RDA (cDNA representational difference analysis) study was performed by using mRNA from AML cells of a patient without cytogenetic abnormalities as the driver and mRNA from AML+8 (trisomy 8) cells of a patient as the tester. cDNA-RDA analysis was performed according to protocols provided by D. G. Schatz (Yale Univ., New Haven, Conn.) and M. J. O'Neill (Princeton

University Princeton) with some modifications. Briefly, 20 mg of total RNA (pooled from AML+8 from three patients for the tester and from cytogenetically normal AML from four patients for the driver) was poly(A)-selected and transcribed into cDNA by using Dynabeads Oligo dT₂₅ (Dynal, Great Neck, N.Y.) and Superscript II reverse transcriptase (Invitrogen). The resulting cDNA was DpnII-digested (New England Biolabs), R-adapters were added with T4 ligase (New England Biolabs), and tester and driver representations were amplified by using Expand High-Fidelity DNA polymerase (Roche Diagnostics). R-adapters were removed by DpnII digest. This step concluded the processing of the driver. For the tester, J-adapters were added by ligation and, the first round of subtractive hybridization was performed at a ratio of 100:1 (driver:tester). Two rounds of PCR with an intermediate mung-bean nuclease digest (New England Biolabs) were performed for difference product 1 (DP1). For a second round of subtractive hybridization, the J-adapters were replaced by N-adapters, and the ratio was increased to 800:1. DP2 was cloned into the BamHI site of pZERO-1 (Invitrogen). Inserts were PCR-amplified by using M13 universal primers and sequenced on an ABI 377 sequencer (Applied Biosystems). Sequences were used in BLAST searches against GenBank.

The sequencing, as above, of 209 RDA clones detected a total of 27 genes, 5 ESTs, and 5 unknown sequences. One frequently detected sequence was EST clone AA400649, belonging to Uni-Gene cluster Hs.169395 in chromosome 8q22.3. This gene was named BAALC for brain and acute leukemia, cytoplasmic.

RDA clone sequences representing partial cDNA clones from BAALC were used to design PCR primers, which were labeled and used in reverse transcription (RT)-PCR using a subset of the human bacterial artificial chromosome (BAC) library RPC-11 as template. Four positive BAC clones, RP11-754L23, RP11-626K19, RP11-701N2, and RP11-773L13 were found. Primer walking, cDNA clone sequencing, and RT-PCR allowed assemblage of the BAALC gene (GenBank accession no. AF363578, which also contains the genomic sequence of BAALC), which is fully contained in BAC clone RP11-754L23 (on human chromosome 8) (FIG. 1). All remaining gaps in BAC clones 680F3 and 318M2 (GenBank AC025936 and AC025370) were closed by primer walking. BAALC covers 90 kb of genomic sequence. Exon 1 is located 72 kb distal to the 3'-end (D8S1610) of the ATP6C gene, and exon 8 (D8S1977) is 68 kb proximal to the 5'-end of the FZD6 gene. The DNA sequences of exons 1-8 are shown in FIG. 2. Using a similar strategy, mouse Baalc was also cloned.

BAALC is Conserved in Mammals

Database searches for BAALC orthologs in other species detected ESTs from mouse (UniGene Mm.44234 and Mm.85430) and rat (Rn.19969 and Rn.57689). In addition, three domestic pig ESTs (BF190130, BF192691, and BF193189) when assembled, contained the complete orthologous ORF for isoform 1-6-8 (see below). The putative 145-amino acid protein was 92% conserved compared with the human. Deposited mouse ESTs predicted that the mouse expressed orthologous transcripts to human BAALC 1-6-8 and 1-8 (see below). The predicted protein isoform 1-6-8 was 89% conserved compared with the human. RT-PCR was performed on brain RNA from human, domestic pig, rat, and mouse and confirmed the expression of both orthologous transcripts 1-6-8 and 1-8 in all species (FIG. 3E). Rat isoform 1-6-8 was highly similar (98%) to the mouse and was also 89% conserved compared with the human. Moreover, pig, mouse, and rat isoforms 1-8 were all 95% conserved compared with human (GenBank accession nos. AF371319-

AF371326). A mouse multitissue Northern blot showed clear preference for the second polyadenylation signal, with high expression of a 2.7-kb transcript in brain (FIG. 4C). Finally, the mouse Baalc gene locus was mapped with the T31 radiation hybrid panel to proximal mouse chromosome 15. The data had the highest anchor logarithm of odds (lod) score of 15.6 to D15Mit112, then lod score 14.4 to D15Mit6, and finally lod score 11.5 to D15Mit22. The best-fit location was between D15Mit112 proximal and D15Mit85 distal.

BAALC Expression is Specific for Neuroectoderm-Derived Tissues

The expression pattern of BAALC in human and mouse was investigated. Most ESTs from BAALC (Uni-Gene Hs.169395) were from central nervous system (CNS)-derived cDNA libraries, and exon 6 was found to be alternatively spliced in brain RNA (FIG. 3A). Thus, the gene was expressed in two transcripts, transcript 1-6-8 (2827 bp) and transcript 1-8 (2660 bp) with predicted ORFs of 145- and 54-amino acids, respectively (FIG. 1B).

By comparative RT-PCR, bone marrow (BM) showed barely detectable, and peripheral blood leukocytes (PBL) showed no expression of BAALC (FIG. 3A). BAALC expression in BM was confined to the CD34-positive progenitor cells.

The tissue specificity of BAALC was examined using multitissue Northern (MTN) blots were assayed by using transcript 1-6-8 as a probe (FIG. 4A). Human BAALC transcript 1-6-8 and mouse Baalc transcript 1-6-8 were random primer-labeled (Roche Diagnostics) and used to probe species-specific multi-tissue Northern blots (CLONTECH). All blots were probed with human β -actin cDNA as a control.

The results of this study showed that BAALC expression was specific for neuroectoderm-derived tissues. BAALC showed high expression in all CNS tissues, including the spinal cord. Moderate levels of BAALC expression were found in adrenal gland and weak levels in thyroid and spleen, which are in part neuroectoderm-derived. In other tissues, notably BM, PBL, and lymph nodes, no detectable levels of BAALC were recorded (FIG. 4A). Furthermore, an MTN blot containing eight human cancer cell lines tested completely negative for BAALC. In addition, an MTN blot containing eight human cancer cell lines (promyelocytic leukemia HL-60, cervix adenocarcinoma HeLa S3, chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361), tested completely negative for BAALC (FIG. 4B). All cell lines were obtained from American Type Culture Collection. These Northern blot experiments showed the expression of two major transcripts of 2 kb and 3 kb with a third minor transcript of about 1 kb, indicating alternative usage of the three observed polyadenylation signals in exon 8, but without affecting the putative ORFs.

Alternative BAALC Transcripts and Protein Isoforms

As described above, alternatively spliced transcripts 1-6-8 and 1-8 are encoded by BAALC. Six additional transcripts, 1-5-6-8, 1-4-5-6-8, 1-5-6-7-8, 1-2-6-8, 1-2-5-6-8 and 1-2-3-6-8 also exist and are most often seen in certain tumor or cancer cells (see Example 1 below). These transcripts arise from alternative splicing and comprise combinations of the exons shown in FIG. 2. These transcripts are shown schematically in FIG. 1B and the sequences of the transcripts are shown in FIGS. 5-12.

Translation of the 8 different transcripts of BAALC indicated 6 different protein isoforms. The amino acid sequences of proteins encoded by each of the alternatively spliced tran-

scripts are shown in FIG. 15. All have the same first 53 amino acids encoded by exon 1, but the remaining coding sequences are different. The use of exon 2 in 3 of the transcripts indicated the same 80-amino acid protein was encoded, as the sequence of exon 2 comprises a termination codon. The coding sequences of all transcripts, except 1-2-3-6-8, were cloned into an expression vector that added 63 amino acids, containing 5 Myc epitopes, to the N terminus of all isoforms. After the clones were expressed in 293 cells, the resulting proteins were immunoprecipitated with anti-Myc antibody and assayed for BAALC expression by Western blot analysis. Protein expression for isoforms 1-6-8 and 1-8, and for the 3 isoforms 1-5-6-8, 1-4-5-6-8, and 1-5-6-7-8 were detected (FIG. 13A). To study the intracellular localization of the proteins, NIH/3T3 cells were transfected with expression constructs from all five transcripts noted above. Using fluorescence microscopy, all isoforms were localized to the cytoplasm, where they appeared as a few large inclusions in the cellular periphery (FIG. 14A-C).

BAALC Expression and Overexpression in Cancers

Methods are provided to determine the level of BAALC gene expression in a biological sample. One method determines the amount of RNA transcribed from the BAALC gene. Another method determines the amount of BAALC protein.

Isolation of RNA from Patient Cell Samples

To determine the amount of BAALC RNA in a sample, RNA is first isolated from the tissue or cells comprising the biological sample. The biological sample is preferably a peripheral blood sample as was described earlier. A variety of methods of RNA isolation from cells and/or tissues is well known to those skilled in the art. Any of such methods can be used. One such method uses the Trizol® reagent from Gibco BRL. Such methods isolate total cellular RNA. Other methods isolate polyadenylated RNA. Methods that provide either type of RNA can be used.

RT-PCR

Reverse transcriptase reactions coupled to polymerase chain reactions (RT-PCR) is one method to assay for the presence of an RNA in a pool of total RNA from a tissue or cell. Detection of a particular RNA is dependent on primers used in the PCR reaction.

RT

The initial step in RT-PCR is a reverse transcription step. Procedures for reverse transcription are well known to those skilled in the art and a variety of procedures can be used. Either total RNA or polyadenylated mRNA can be used as the template for synthesis of cDNA by the reverse transcriptase enzyme.

In one embodiment, oligo(dT) is used as the primer in the reverse transcription reaction. Oligo(dT) hybridizes to the poly(A) tails of mRNAs during first strand cDNA synthesis. Since all mRNAs normally have a poly(A) tail, first strand cDNA is made from all mRNAs present in the reaction (i.e., there is no specificity). In another embodiment, specific primers are used in place of oligo(dT) and specific RNAs are reverse transcribed into DNA. The specific primers preferably are complementary to a region near the 3' end of the RNA in order that full length or nearly full length cDNA is produced. Primer selection is preferably made using the guidelines described below for selection of PCR primers. A number of different primers can be used with good results. For reverse transcription of BAALC RNA, two different primers are preferably alternatively used. The first primer is called ES99 and has the sequence 5'-CATCTGTTGGATGCTATCTG-3' (SEQ ID NO:23). The second primer is called ES10 and has the sequence 5'-TGGACTCTCTGCTAGTTGAC-3' (SEQ ID

NO:24). For reverse transcription of the housekeeping gene, glucose phosphate isomerase (GPI) the primer preferably is called GPI Exon3R and has the sequence 5'-TCGGTGTAGT-TGATCTTCTC-3' (SEQ ID NO:25).

Preferably, the reverse transcriptase enzyme used in the reaction is stable at temperatures above 60° C., for example, SuperScript II RT (Gibco BRL). However, MMLV reverse transcriptase can also be used. In one embodiment, the reverse transcriptase reaction mixture contains 10 mM Tris (pH 8.3), 40 mM KCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 200 μM each of dATP, dCTP, dTTP and dGTP, 200 ng of the primer, 10 U of AMV reverse transcriptase from Boehringer Mannheim Biochemicals, and 20 units of RNASIN from Promega.

The disaccharide, trehalose, can be added to the reverse transcriptase reaction. Trehalose is a disaccharide that has been shown to stabilize several enzymes including RT at temperatures as high as 60° C. (Mizuno, et al., *Nucleic Acids Res.* 27:1345-1349, 1999). Trehalose addition allows the use of high temperatures in the reverse transcription reaction (e.g., as high as 60° C.). Therefore, trehalose can be added to the reverse transcriptase reaction such that it is present in a final concentration of between 20 to 30%. Preferably, the reverse transcriptase reaction is then performed at a temperature between 35 to 75° C., more preferably at a temperature from between 50 to 75° C., most preferably at a temperature of 60° C.

PCR

Once the reverse transcriptase reaction is carried out, the cDNA produced is amplified by PCR. In one embodiment, the entire RT-PCR reaction is carried out on a standard thermal cycler according to the methods described in the GeneAmp RNA PCR kit obtained from Perkin-Elmer/Cetus, for example. A 0.5 pg sample of total RNA from the cells is used to produce the first strand cDNA. The amplification cycle protocol is as follows: 95° C. for 2 minutes, 95° C. for 1 minute, 56° C. for 1 minute, and 72° C. for 2 minutes, through 35 cycles.

In another embodiment, a standard PCR reaction contains a buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 6.0 mM MgCl₂, 200 μM each of dATP, dCTP, dTTP and dGTP, two primers of concentration 0.5 μM each, 7.5 ng/ul concentration of template cDNA and 2.5 units of Taq DNA Polymerase enzyme. Variations of these conditions can be used and are well known to those skilled in the art.

The PCR reaction is preferably performed under high stringency conditions. Herein, "high stringency PCR conditions" refers to conditions that do not allow base-pairing mismatches to occur during hybridization of primer to template. Such conditions are equivalent to or comparable to denaturation for 1 minute at 95° C. in a solution comprising 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 6.0 mM MgCl₂, followed by annealing in the same solution at about 62° C. for 5 seconds.

The products of the PCR reaction can be detected in various ways. One way is by agarose gel electrophoresis which involves separating the DNA in the PCR reaction by size in electrophoresis. The agarose gel is then stained with dyes that bind to DNA and fluoresce when illuminated by light of various wavelengths. Preferably the dye used is ethidium bromide and the illumination uses an ultraviolet light.

Primer Selection

One primer is located at each end of the region to be amplified. Such primers will normally be between 10 to 30 nucleotides in length and have a preferred length from between 18 to 22 nucleotides. The smallest sequence that can be amplified is approximately 50 nucleotides in length (e.g.,

a forward and reverse primer, both of 20 nucleotides in length, whose location in the sequences is separated by at least 10 nucleotides). Much longer sequences can be amplified. Preferably, the length of sequence amplified is between 75 and 250 nucleotides in length.

One primer is called the "forward primer" and is located at the left end of the region to be amplified. The forward primer is identical in sequence to a region in the top strand of the DNA (when a double-stranded DNA is pictured using the convention where the top strand is shown with polarity in the 5' to 3' direction). The sequence of the forward primer is such that it hybridizes to the strand of the DNA which is complementary to the top strand of DNA.

The other primer is called the "reverse primer" and is located at the right end of the region to be amplified. The sequence of the reverse primer is such that it is complementary in sequence to a region in the top strand of the DNA. The reverse primer hybridizes to the top strand of the DNA.

PCR primers should also be chosen subject to a number of other conditions. PCR primers should be long enough (preferably 10 to 30 nucleotides in length) to minimize hybridization to greater than one region in the template. Primers with long runs of a single base should be avoided, if possible. Primers should preferably have a percent G+C content of between 40 and 60%. If possible, the percent G+C content of the 3' end of the primer should be higher than the percent G+C content of the 5' end of the primer. Primers should not contain sequences that can hybridize to another sequence within the primer (i.e., palindromes). Two primers used in the same PCR reaction should not be able to hybridize to one another. Although PCR primers are preferably chosen subject to the recommendations above, it is not necessary that the primers conform to these conditions. Other primers may work, but have a lower chance of yielding good results.

PCR primers that can be used to amplify DNA within a given sequence are preferably chosen using one of a number of computer programs that are available. Such programs choose primers that are optimum for amplification of a given sequence (i.e., such programs choose primers subject to the conditions stated above, plus other conditions that may maximize the functionality of PCR primers). One computer program is the Genetics Computer Group (GCG recently became Accelrys) analysis package which has a routine for selection of PCR primers. There are also several web sites that can be used to select optimal PCR primers to amplify an input sequence. One such web site is alces.med.umn.edu/rawprimer.html. Another such web site is www-genome.wi.mit.edu/cgi-in/primer/primer3_www.cgi.

Forward and reverse primers can be selected from a variety of regions of the BAALC gene. Actually, a very large number of primers can be designed using the sequence of the BAALC gene and such probes successfully used. Preferably, for PCR amplification of BAALC, the forward primer is designed using a sequence within exon 6 of BAALC and the reverse primer is designed using a sequence within exon 8 of BAALC. Both the forward and reverse primers can also be designed using sequences within exon 8.

Three different sets of primers are preferably used alternatively for BAALC. Primer set 1 produces a 173 base pair amplification product. The set 1 primers are the forward primer (ES6) 5'-ACCCAGAGAAGAAGACGAAC-3' (SEQ ID NO:26) and the reverse primer (ES99) 5'-CATCTGTTG-GATGCTATCTG-3' (SEQ ID NO:23). Primer set 2 produces a 101 base pair amplification product. The set 2 primers are the forward primer (ES9) 5'-AGAAACAGAATGGCCT-TCAG-3' (SEQ ID NO:27), and the reverse primer (ES99) 5'-CATCTGTTGGATGCTATCTG-3' (SEQ ID NO:23).

Primer set 3 produces a 75 base pair amplification product. The set 3 primers are the forward primer (BAALC 6F) 5'-GC-CCTCTGACCCAGAAACAG-3' (SEQ ID NO:28), and the reverse primer (BAALC 8R) 5'-CTTTTGCAGGCAT-TCTCTTAGCA-3' (SEQ ID NO:29).

The GPI primers preferably used are the forward primer (GPI Exon1F) 5'-TCTTCGATGCCAACAAGGAC-3' (SEQ ID NO:30), and the reverse primer (Hsa E2R) 5'-GCAT-CACGTCCTCCGTCAC-3' (SEQ ID NO:31).

Real-Time PCR

The PCR procedure can also be done in such a way that the amount of PCR products can be quantified. Such "quantitative PCR" procedures normally involve comparisons of the amount of PCR product produced in different PCR reactions. A number of such quantitative PCR procedures, and variations thereof, are well known to those skilled in the art. One inherent property of such procedures, however, is the ability to determine relative amounts of a sequence of interest within the template that is amplified in the PCR reaction.

One particularly preferred method of quantitative PCR used to quantify copy numbers of sequences within the PCR template is a modification of the standard PCR called "real-time PCR." Real-time PCR utilizes a thermal cycler (i.e., an instrument that provides the temperature changes necessary for the PCR reaction to occur) that incorporates a fluorimeter (i.e. an instrument that measures fluorescence). In one type of real-time PCR, the reaction mixture also contains a reagent whose incorporation into a PCR product can be quantified and whose quantification is indicative of copy number of that sequence in the template. One such reagent is a fluorescent dye, called SYBR Green I (Molecular Probes, Inc.; Eugene, Oreg.) that preferentially binds double-stranded DNA and whose fluorescence is greatly enhanced by binding of double-stranded DNA. When a PCR reaction is performed in the presence of SYBR Green I, resulting DNA products bind SYBR Green I and fluoresce. The fluorescence is detected and quantified by the fluorimeter. Such technique is particularly useful for quantification of the amount of template in a PCR reaction.

A preferred variation of real-time PCR is TaqMan® (Applied Biosystems) PCR. The basis for this method is to continuously measure PCR product accumulation using a dual-labeled fluorescent oligonucleotide probe called a TaqMan® probe. The "probe" is added to and used in the PCR reaction in addition to the two primers. This probe is composed of a short (ca. 20-30 bases) oligodeoxynucleotide sequence that hybridizes to one of the strands that are made during the PCR reaction. That is, the oligonucleotide probe sequence is homologous to an internal target sequence present in the PCR amplicon. The probe is labeled or tagged with two different fluorescent dyes. On the 5' terminus is a "reporter dye" and on the 3' terminus is a "quenching dye." One reporter dye that is used is called 6-carboxy fluorescein (FAM). One quenching dye that is used is called 6-carboxy tetramethyl-rhodamine (TAMRA). When the probe is intact, energy transfer occurs between the two fluorochromes and emission from the reporter is quenched by the quencher, resulting in low, background fluorescence. During the extension phase of PCR, the probe is cleaved by the 5' nuclease activity of Taq polymerase, thereby releasing the reporter from the oligonucleotide-quencher and producing an increase in reporter emission intensity. During the entire amplification process the light emission increases exponentially.

Although a variety of different probes can be used, the preferred probes are as follows: the probe used with the BAALC set 1 and 2 PCR primers is TaqExonD with the sequence 5'-CAGGCATTCTCTTAGCATCTCTTTT-3'

(SEQ ID NO:32). The probe used with the BAALC set 3 PCR primers is 5'-CTCTTTTAGCCTCTGTGGTCTGAAGGC-CAT-3' (SEQ ID NO:33). The probe used with the previously described probes for amplification of GPI (GPI Exon1F and Has E2R) has the sequence 5'-TTCAGCTTGACCCTCAACACCAAC-3' (SEQ ID NO:34).

The instrument used to detect the fluorescence is preferably an ABI Prism 7700, which uses fiber optic systems that connect to each well in a 96-well PCR tray format. The laser light source excites each well and a CCD camera measures the fluorescence spectrum and intensity from each well to generate real-time data during PCR amplification. The ABI 7700 Prism software examines the fluorescence intensity of reporter and quencher dyes and calculates the increase in normalized reporter emission intensity over the course of the amplification. The results are then plotted versus time, represented by cycle number, to produce a continuous measure of PCR amplification. To provide precise quantification of initial target in each PCR reaction, the amplification plot is examined at a point during the early log phase of product accumulation. This is accomplished by assigning a fluorescence threshold above background and determining the time point at which each sample's amplification plot reaches the threshold (defined as the threshold cycle number or CT). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube as described in the Examples which follow below.

Northern Blotting

In addition to RT-PCR, other procedures can be used to detect RNA that is transcribed from the BAALC gene. One such method is known as Northern blot hybridization. In this method, RNA is isolated from tissues or cells and separated by size using gel electrophoresis. The RNAs in the gel are then transferred to a membrane. After transfer of the RNA to the membrane, a nucleotide probe is labeled and hybridized to the RNA on the membrane. Hybridization of a DNA probe to RNA on the membrane is detected by autoradiography or chemiluminescence.

A variation of Northern blotting, is called slot blotting or dot blotting. In this technique, the isolated RNA is applied directly to a membrane. The nucleotide probe is then labeled and hybridized to the RNA on the membrane. Hybridization is detected by autoradiography or chemiluminescence.

Probes of many different lengths and sequences can be designed and used in Northern blotting experiments to detect BAALC transcripts.

Antibodies

Another method for detecting and quantifying overexpression of BAALC uses antibodies immunospecific for one or more isoforms of the BAALC protein to detect the protein in extracts or fixed cells, for example. Hereinafter, unless specifically indicated otherwise, "BAALC protein" refers not to a single protein, but to multiple BAALC proteins that are represented by the different isoforms (see FIG. 15). The present invention provides antibodies that are immunospecific for the BAALC protein. As used herein, the term "immunospecific" means the antibodies have greater affinity for the BAALC protein than for other proteins. Preferably, the affinity of the antibodies for BAALC protein is many fold greater than their affinity for any other proteins. Most preferably, the BAALC antibodies do not have affinity for any proteins other than BAALC protein.

The term "antibody" encompasses monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity or specificity. "Antibody fragments" comprise a portion of a full length antibody,

15

generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments.

Antibodies raised against BAALC are produced by immunizing a host animal with a BAALC protein or an antigenic fragment thereof. Suitable host animals for injection of the protein immunogen include, but are not limited to, rabbits, mice, rats, goats, and guinea pigs. Various adjuvants may be used to increase the immunological response of the immunogen or antigen (i.e., the BAALC protein or peptide) in the host animal. The adjuvant used depends, at least in part, on the host species. For example, guinea pig albumin is commonly used as a carrier for immunizations in guinea pigs. Such animals produce heterogeneous populations of antibody molecules, which are referred to as polyclonal antibodies and which may be derived from the sera of the immunized animals. Such sera may be used directly, or the specific antibodies desired can be purified from the sera, using methods well known to those of skill in the art.

Antibodies are also prepared using an oligopeptide having a sequence which is identical to a portion of the amino acid sequence of a BAALC protein isoform. Preferably the oligopeptide has an amino acid sequence of at least five amino acids, and more preferably, at least 10 amino acids that are identical to a portion of the amino acid sequence of a BAALC protein. Preferably, the peptides used are either GN2214, having an amino acid sequence of DAIEPRYYESWTRETEST (SEQ ID NO:35), or GN2216, having an amino acid sequence of DSIQQMDRSRRITK (SEQ ID NO:36). SEQ ID NO:35 is found in all 6 of the BAALC protein isoforms shown in FIG. 15. SEQ ID NO:36 is found in 2 of the BAALC protein isoforms shown in FIG. 15, namely the 1-6-8 and 1-5-6-8 isoforms. Such peptides are conventionally fused with those of another protein such as keyhole limpet hemocyanin and antibody is produced against the chimeric molecule. Such peptides can be determined using software programs, for example the MacVector program, to determine hydrophilicity and hydrophobicity and ascertain regions of the protein that are likely to be present at the surface of the molecule.

The term "monoclonal antibody" as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site, also called epitope. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method, first described by Kohler and Milstein (Nature 256:495-497, 1975), in which case the hybridoma cell lines that are obtained secrete the monoclonal antibodies during growth. As is known in the art, hybridomas that secrete monoclonal antibodies are made by injecting mice with the desired antigen. The antigens frequently are peptide antigens which are chosen using similar procedures as described above for selection of peptide antigens for making polyclonal antibodies. Although many different peptide antigens from BAALC likely give good results, a preferred peptide antigen for making monoclonal antibodies has the amino acid sequence RADAIEPRYYESWTRETESTWLTYT (SEQ ID NO:37). SEQ ID NO:37 is found in all 6 of the BAALC protein

16

isoforms shown in FIG. 15. After the antigens have been injected into the mice, spleen cells are taken from the immunized mice and are fused to myeloma cells. Clones of fusion cells are then obtained and are screened for production of anti-BAALC antibodies.

Various immunoassays may be used for screening to identify antibodies having the desired specificity. These include protocols which involve competitive binding or immunoradiometric assays and typically involve the measurement of complex formation between the respective BAALC protein and the antibody.

In order to grow the hybridoma cell lines and obtain the secreted antibodies, the hybridoma cell lines may be grown in cell culture and culture medium containing the monoclonal antibodies collected. Alternatively, the hybridoma cell lines may be injected into, and grown within, the peritoneal cavity of live animals, preferably mice. As the hybridoma cell lines grow within the peritoneal cavity of the animal, the monoclonal antibodies are secreted. This peritoneal fluid, called "ascites," is collected using a syringe to obtain the monoclonal antibodies. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any class thereof.

Antibody preparations may be isolated or purified. An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody may be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

Antibodies immunospecific for BAALC are useful for identifying tissues or cells that express BAALC proteins. The diagnostic/prognostic methods comprise the steps of contacting tissues or cells with such antibody and assaying for the formation of a complex between the antibodies and a BAALC protein in the samples. Preferably the cells are permeabilized. Interactions between antibodies and a protein or peptide in the sample are detected by radiometric, colorimetric, or fluorometric means. Detection of the antigen-antibody complex may be accomplished by addition of a secondary antibody that is coupled to a detectable tag, such as for example, an enzyme, fluorophore, or chromophore. Preferably, the detection method employs an enzyme-linked immunosorbent assay (ELISA), Western immunoblot procedure and/or immunoprecipitation.

Western Blots

Protein or cell-free extracts are made from tissues or cells. In one method, cells are lysed in 500 μ l ice-cold Lysis Buffer (50 mM Tris pH 7.5; 1% Triton X-100; 100 mM NaCl; 50 mM NaF; 200 μ M Na₃VO₄; 10 μ g/ml pepstatin and leupeptin) (all chemicals from Sigma Chemical Co., St. Louis, Mo.) for approximately 30 min at 4° C. The cell lysate suspension is then microcentrifuged at 4° C. (14,000 RPM for 10 min). The supernatant is removed and stored at -80° C. Proteins are separated using SDS-polyacrylamide gel electrophoresis

17

(SDS-PAGE) through a 6%-7.5% acrylamide gel at 100V. The samples are transferred to an Immobilon-P membrane (Millipore, Bedford, Mass.). Blots are blocked in phosphate buffered saline (PBS: 138 mM NaCl, 15 mM Na₂HPO₄, 1.5 mM KCl, and 2.5 mM KH₂PO₄), containing 5% non-fat dehydrated milk and 0.1% Tween-20 (Sigma Chemical Co., St. Louis, Mo.) overnight at 4° C. Blots are incubated for 90-120 min at room temperature in PBS with primary anti-BAALC antibody and then washed three times in PBS with 0.1% Tween-20. Blots are then incubated with secondary antibody conjugated to horseradish peroxidase (1:4000 dilution) (Sigma Chemical Co., St. Louis, Mo.) for 1 hour at room temperature and washed again as described above. Signal is visualized by incubating with Super Signal chemiluminescent substrate (Pierce, Rockford, Ill.) and exposing the membrane to Kodak scientific imaging film (Kodak, Rochester, N.Y.).

EXAMPLES

Further details of the invention can be found in the following examples, which further define the scope of the invention and serve to illustrate but not to limit the present invention.

Example 1

Alternative Splicing of BAALC in Acute Myeloid Leukemia Samples

Blasts from BM or peripheral blood (PB) were collected from patients with AML. Total RNA was isolated using Trizol (Invitrogen). Total RNA from AML cells from these patients was reverse-transcribed with avian myeloblastosis virus reverse transcriptase (Roche Diagnostics) using oligo(dT) as primer. The reverse transcriptase reaction mixture contained 10 mM Tris (pH 8.3), 40 mM KCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 200 μM each of dATP, dCTP, dTTP and dGTP, 200 ng of the primer, 10 U of AMV reverse transcriptase from Boehringer Mannheim Biochemicals, and 20 units of RNA-SIN from Promega. The reverse transcriptase reaction was then performed at a temperature of 60° C.

PCR was then performed using the cDNA from the reverse transcriptase reaction as template. Primer sequences from exons 1 and 8 of human BAALC were used. The primer from exon 1 was ES100, 5'-GTGCGGTACCAAGCTTCCGCG-GCGCAGGAGGATG-3' (SEQ ID NO:38). The primer from exon 8 was ES102, 5'-CGGGGTACCGTTGACACAGT-TCTTTGTGATTC-3' (SEQ ID NO:39). Both BAALC and the housekeeping gene GPI (glucose phosphate isomerase) were coamplified in the same tube. The primer sequences for GPI were the forward primer (GPI Exon1F) 5'-TCTTCGAT-GCCAACAAGGAC-3' (SEQ ID NO:30) and the reverse primer (Hsa E2R) 5'-GCATCACGTCCTCCGTCAC-3' (SEQ ID NO:31). The PCR reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 6.0 mM MgCl₂, 200 μM each of dATP, dCTP, dTTP and dGTP, the two primers of concentration 0.5 μM each, 7.5 ng/ul concentration of template cDNA and 2.5 units of Taq DNA Polymerase enzyme. The amplification cycle protocol was as follows: 95° C. for 2 minutes, 95° C. for 1 minute, 56° C. for 1 minute, and 72° C. for 2 minutes, through 35 cycles. The products of the PCR reaction were then separated by agarose gel electrophoresis and the gel was stained with ethidium bromide, illuminated with ultraviolet light, and photographed.

The results (FIG. 3B) showed that transcripts were detected in the AML samples that were not seen previously in the CNS. The PCR fragments were cloned and sequenced

18

using standard techniques showed the presence of 5 new exons that were numbered 2-5 and 7, which led to 6 additional alternatively spliced transcripts (FIG. 1B). Including the transcripts 1-6-8 and 1-8, there were 8 alternatively spliced transcripts (FIG. 1B and FIGS. 5-12) in the blasts of patients with acute leukemia who were expressing high levels of BAALC.

Additional studies showed that 5 of 27 diverse patients with AML contained elevated or overexpressed BAALC transcript levels as compared to GPI (e.g., see lanes 1 and 2 in FIG. 3C). Certain of the AML samples expressed very high levels of BAALC. FIG. 3B shows this to be true for AML+8 nos. 1 and 3.

Example 2

BAALC Overexpression in Normal Cells and Various Cancer Samples, Including Glioblastoma Samples

Real-time PCR (TaqMan®, Applied Biosystems) was used to quantify the relative levels of BAALC transcripts in various tissues and cancers using total RNA isolated from the cells using Trizol (Invitrogen). Initially, RNA samples from several organs including BM, PBL, brain and fetal brain, plus different human tumor samples were analyzed. Additionally, RNA samples from 10 normal human tissues, 10 colorectal cancer-normal colonic mucosa-matched sample pairs, 3 esophageal cancer-normal esophagus-matched sample pairs, 3 lung tumor samples, 5 glioblastoma samples, 2 thyroid carcinoma-normal thyroid-matched sample pairs, 1 thyroid carcinoma sample, and 3 testicular and 3 mammary tumor samples were analyzed for expression of BAALC.

Reverse transcription was performed using the total RNA isolated from the cells. Separate primers for reverse transcription were used to synthesize cDNA for both BAALC and for GPI. For BAALC, two primers were alternatively used: (ES99) 5'-CATCTGTTGGATGCTATCTG-3' (SEQ ID NO:23) or (ES10) 5'-TGGACTCTCTGCTAGTTGAC-3' (SEQ ID NO:24). Both ES99 and ES10 were derived from sequences within exon 8 of BAALC. For GPI, the primer (GPI Exon3R) was 5'-TCGGTGTAGTTGATCTTCTC-3' (SEQ ID NO:25).

Real-time PCR was then performed with both the BAALC and GPI housekeeping gene coamplified in the same tube. The TaqMan® real-time PCR system (Applied Biosystems) was used. Separate primers and probes were used for both BAALC and for GPI.

Three different sets of primers were alternatively used for BAALC. Primer set 1 produced a 173 base pair amplification product. The set 1 primers were the forward primer (ES6) 5'-ACCCAGAGAAGAAGACGAAC-3' (SEQ ID NO:26) and the reverse primer (ES99) 5'-CATCTGTTGGATGCTATCTG-3'. ES6 was derived from sequences within exon 6 of BAALC. The BAALC probe (TaQExonD) used with set 1 primers was 5'-CAGGCATTCTCTTAGCATCTCTTTT-3' (SEQ ID NO:32). Primer set 2 produced a 101 base pair amplification product. The set 2 primers were the forward primer (ES9) 5'-AGAAACAGAATGGCCTTCAG-3' (SEQ ID NO:27), and the reverse primer (ES99) 5'-CATCTGTTGATGCTATCTG-3' (SEQ ID NO:23). ES9 was derived from sequences within exon 6 of BAALC. The BAALC probe (TaQExonD) used with set 2 primers was 5'-CAGGCATTCTCTTAGCATCTCTTTT-3' (SEQ ID NO:32). Primer set 3 produced a 75 base pair amplification product. The set 3 primers were the forward primer (BAALC 6F) 5'-GC-CCTCTGACCCAGAAACAG-3' (SEQ ID NO:28), and the reverse primer (BAALC 8R) 5'-CTTTTGCAGGCAT-

19

TCTCTTAGCA-3' (SEQ ID NO:29). BAALC 6F was derived from sequences within exon 6 of BAALC. BAALC 8R was derived from sequences within exon 8 of BAALC. The BAALC probe used with set 3 primers was 5'-CTCTTT-TAGCCTCTGTGGTCTGAAGGCCAT-3' (SEQ ID NO:33). 5

The GPI primers and probes were the forward primer (GPI Exon1F) 5'-TCTTCGATGCCAACAAGGAC-3' (SEQ ID NO:30), and the reverse primer (Hsa E2R) 5'-GCAT-CACGTCCTCCGTCAC-3' (SEQ ID NO:31). The GPI probe used with the GPI primers was 5'-TTCAGCTTGACCCT-CAACACCAAC-3' (SEQ ID NO:34). The analysis was performed using triplicate samples.

To determine the relative levels of expression of BAALC among the samples, the comparative C_T method was used (Applied Biosystems). Briefly, the threshold cycles (C_T) for BAALC and Glucose-phosphate isomerase (GPI) were determined, and the cycle number difference ($\Delta C_T = \text{GPI} - \text{BAALC}$) was calculated for each replicate. If BAALC failed to reach the software-set threshold, the sample was considered below detection limit. If GPI amplification failed, the sample was omitted from the analysis. Finally, relative BAALC expression was calculated using the mean of ΔC_T from the three replicates, that is $MC_T = \mu(\Delta C_T) = (\sum \Delta C_T)/3$, normalizing BAALC expression to GPI expression.

Aside from the AML samples (Example 1) already scored as overexpressing BAALC, only glioblastoma tumor samples showed comparable levels of BAALC expression. All five glioblastoma samples strongly expressed BAALC in the range of 0.74-3.71 MC_T , but only transcripts 1-6-8 and 1-8 (FIG. 3D), distinguishing them from the acute leukemia samples (FIG. 3C).

A cut off of $MC_T \geq 0.75$ was used to score leukemia samples as overexpressors. The MC_T for normal tissues, including brain, fetal brain, spleen, and several normal BM samples were well below this threshold and in the range of -12.4 to -0.14 MC_T . Because low expression in five BM samples was obtained (-10.57 to -7.2 MC_T), CD34-positive progenitor cells of two healthy donors were twice immunomagnetically enriched. The >95% pure CD34-positive hematopoietic progenitor populations expressed BAALC in the range of -4.2 to -2.0 MC_T , whereas the CD34-negative fractions were below the detection limit.

Example 3

BAALC Overexpression in Leukemia Samples

Real-time PCR (TaqMan®, Applied Biosystems) was used to quantify the relative levels of BAALC transcripts in different leukemia samples, using total RNA isolated from the cells in real-time PCR, as described in Example 2. Blasts from BM or peripheral blood from 130 diverse AML patients, 31 ALL patients, 4 Burkitt's lymphoma (BL) patients, 5 chronic myelogenous leukemia (CML) patients, 5 chronic lymphocytic leukemia (CLL) patients, plus the 7 leukemia cell lines HL-60, KG-1, KG-1a, MC-1010, K-562, D1.1, and RS4;11, were analyzed. Pretreatment BM aspirate or peripheral blood samples from patients with the different leukemias were collected after prior consent. CD34-positive progenitor cells were enriched twice from normal BM aspirates by immunomagnetic separation, using MiniMACS columns (Miltenyi Biotec, Auburn, Calif.). Cell lines were obtained from the American Type Culture Collection. Total RNA was isolated using Trizol (Invitrogen).

Cases with $MC_T \geq 0.75$ were classified as BAALC overexpressors. In AML, 37/130 (28%), and in ALL, 20/31 (65%) were classified as BAALC overexpressors (0.75 to 8.59

20

MC_T), but none of the BL, CML, and CLL samples, or leukemia cell lines scored as overexpressors (below detection limit to 0.46 MC_T).

Example 4

BAALC Overexpression in AML Samples

Real-time PCR (TaqMan®, Applied Biosystems) was used to quantify the relative levels of BAALC transcripts in samples from patients with AML, using total RNA isolated from the cells in real-time PCR, as described in Example 2. One hundred two samples from adult patients with de novo AML from which peripheral blood samples were available and centrally reviewed clinical data were statistically analyzed. The samples were obtained from the Cancer and Leukemia Group B (CALGB) Leukemia Tissue Bank (see Proc. Natl. Acad. Sci. 98:13901-13906, 2001) and were cancers with evaluable cytogenetics enrolled in a prospective cytogenetic companion study, CALGB 8461. These patients had peripheral blood samples with >50% blasts. Cases with AML with $MC_T \geq 0.75$ were classified as BAALC overexpressors. Event-free survival was measured from the on-study date until date of treatment failure, relapse, or death, censoring only for patients alive and in continuous complete remission.

Of 102 patient samples analyzed by real-time PCR, 29 (28%) were BAALC overexpressors. The distribution of BAALC overexpression differed significantly among AML French-American-British subtypes ($P < 0.0001$). It was overexpressed in all 5 cases of M0, in 12/28 M1, 7/28 M2, and 3/4 M4Eo samples, but in no M3 (0/6), and only in 1/14 M4 and 1/17 M5 samples. The association with cytogenetic subtype was also nonrandom ($P = 0.0001$). BAALC overexpression was seen in 2/2 inv(3)/t(3;3), 6/8 core binding factor leukemia samples, and 3/4 isolated trisomy 8 samples, but in 0/6 samples of t(15;17) and only 1/9 samples of t(11q23). Of 63 (19%) patients, 12 with normal karyotypes overexpressed BAALC. Overexpression of BAALC was an adverse prognostic factor. Among the 29 BAALC overexpressing patients, the median of event-free survival was 0.4 years compared with 1.2 years for the 73 patients that did not overexpress BAALC ($P = 0.006$).

Example 5

BAALC Overexpression in AML Samples with Normal Cytogenetics

Real-time PCR (TaqMan®, Applied Biosystems) was used to quantify the relative levels of BAALC transcripts in AML samples with normal cytogenetics, using total RNA isolated from the cells in real-time PCR, as described in Example 2.

Patients evaluated were adults, diagnosed with de novo AML, enrolled in CALGB treatment protocol 9621 (see Proc. Natl. Acad. Sci. 98:13901-13906, 2001), and enrolled in a prospective cytogenetic companion study, CALGB 8461, with normal cytogenetics, more than 50% peripheral blasts, and sufficient RNA. All patients gave informed consent for treatment and blood samples. Patients received induction chemotherapy consisting of cytarabine, daunorubicin, and etoposide with or without the multi-drug resistance protein modulator, PSC-833. For patients with normal cytogenetics, this was followed by autologous peripheral blood stem cell transplantation (PBSCT). Maintenance therapy consisted of low-dose interleukin 2 interrupted with intermediate dose pulsing of interleukin 2.

Morphology according to the French-American-British (FAB) classification and karyotypes were centrally reviewed by CALGB. The FLT3 genotype was determined in 50 of the 51 AML patients. In addition, 35 of the 51 samples were analyzed for the partial tandem duplication (PTD) of the MLL gene.

Mononuclear cells from pretreatment blood samples were enriched by Ficoll-Hypaque gradient and frozen in liquid nitrogen. Control blood samples were obtained from 10 healthy, adult volunteers. Total RNA was extracted using Trizol (Invitrogen), and comparative real-time RT-PCR (Taq-Man®) was performed in triplicate as previously described in Example 2 above. Briefly, the threshold cycles (CT) for BAALC and Glucose-phosphate isomerase (GPI) were determined, and the cycle number difference ($\Delta CT = GPI - BAALC$) was calculated for each replicate. Relative BAALC expression was calculated using the mean of ΔCT from the three replicates, that is $\mu(\Delta CT) = (\sum \Delta CT)/3$, and expressed as $2^{\mu(\Delta CT)}$. BAALC expression among 10 healthy donors ranged from 0 to 0.0042 with a mean of 0.00072 ± 0.00151 standard deviation (SD). A threshold level of 0.00525 (mean+3×SD) was used to dichotomize AML samples: samples were considered non-overexpressing for BAALC if $2^{\mu(\Delta CT)}$ was ≤ 0.00525 ($MCT \leq -7.57$); samples were considered overexpressing for BAALC if $2^{\mu(\Delta CT)}$ was > 0.00525 ($MC_T > -7.57$).

Samples from fifty-one adult patients were evaluated for BAALC overexpression by real-time RT-PCR. Samples from 7 patients (14%) were BAALC non-overexpressors (range of expression: 0-0.0034), and samples from 44 patients (86%) were BAALC overexpressors with expression ranging from 0.0057 to 8.2630. No significant differences were seen when comparing overexpressing and non-overexpressing patients with respect to age, white blood count (WBC), percentage of blasts, multiple other clinical features including treatment received, frequency of the MLL PTD and the FLT3 genotype (Table 1). Clinical features at presentation were compared using Fisher's Exact test or the Wilcoxon rank-sum test. Inferential tests yielding a p-value < 0.05 were considered to be significant. All BAALC non-overexpressing patients had a wild-type (WT) FLT3^{WT/WT} genotype (n=7), whereas a FLT3 internal tandem duplication (ITD) was present in 20 of the 43 BAALC overexpressing patients. Frequency of FAB subtypes M4/M5 versus other FAB subtypes was significantly lower in BAALC overexpressing patients (30%) compared to BAALC non-overexpressing patients (86%; P=0.009).

TABLE 1

Clinical characteristics at presentation for the BAALC non-overexpressing and BAALC overexpressing AML patients and impact of BAALC expression on clinical outcome.			
	BAALC non-over-expressors n = 7	BAALC over-expressors n = 44	p-value
<u>Age (years)</u>			
Median (Range)	43 (23, 53)	42 (20, 59)	0.98
<u>Hemoglobin (g/dL)</u>			
Median (Range)	8.9 (6.4, 11.8)	9.4 (4.6, 12.9)	0.77
<u>Platelets (x 10⁹/L)</u>			
Median (Range)	50 (12, 144)	54 (8, 235)	0.74

TABLE 1-continued

Clinical characteristics at presentation for the BAALC non-overexpressing and BAALC overexpressing AML patients and impact of BAALC expression on clinical outcome.			
	BAALC non-over- expressors n = 7	BAALC over- expressors n = 44	p-value
<u>WBC (x 10⁹/L)</u>			
Median (Range)	45.5 (17.2, 146)	42.7 (3.7, 295)	0.97
<u>% Peripheral Blasts</u>			
Median (Range)	73 (51, 77)	80 (52, 97)	0.10
<u>% Bone marrow Blasts</u>			
Median (Range)	80 (58, 88)	70 (38, 90)	0.19
<u>FAB classification</u>			
M0	0 (0%)	1 (2%)	0.13
M1	1 (14%)	14 (33%)	
M2	0 (0%)	15 (35%)	
M4	2 (29%)	10 (23%)	
M5	4 (57%)	3 (7%)	
AML unclassifiable		1	
Gum Hypertrophy	3 (43%)	7 (16%)	
Lymphadenopathy	0 (0%)	6 (14%)	
Skin Infiltrates	0 (0%)	6 (14%)	
Hepatomegaly	1 (14%)	3 (7%)	
Splenomegaly	0 (0%)	5 (12%)	1.00
<u>MLL PTD (35 cases analyzed)</u>			
present	0 (0%)	1 (3%)	1.00
absent	6 (100%)	28 (97%)	
<u>FLT3 genotype (50 cases analyzed)</u>			
FLT3 ^{WT/WT}	7 (100%)	23 (53%)	0.11
FLT3 ^{ITD/WT}	0 (0%)	12 (28%)	
FLT3 ^{ITD/-}	0 (0%)	8 (19%)	
Complete Remission	7 (100%)	35 (80%)	0.33
<u>Disease-free Survival</u>			
Median (years)	*	0.7	0.009
% Alive at 2 years (95% CI)	100%	39% (23%, 56%)	
<u>Overall Survival</u>			
Median (years)	*	1.0	0.006
% Alive at 2 years (95% CI)	100%	39% (24%, 53%)	

*Not estimable, all patients censored for the outcome;
CI: confidence interval

Using these data, patient survival was calculated. There was a significant inverse correlation between BAALC overexpression and patient survival. Overall survival (OS) was measured from on-study date until date of death, regardless of cause of death, censoring for patients alive. With at least two years of follow-up, among the 44 BAALC overexpressing patients (overexpressors) the median overall survival was 1.0 year (38% alive at 2 years) compared with all 7 BAALC non-overexpressing patients (100%) being alive at 2 years (censored for the outcome; P=0.006; FIG. 16A). Event-free survival (EFS) was defined for those achieving complete remission (CR) as the time from on-study until relapse or death regardless of cause, censoring for those alive at last follow-up. If a patient did not achieve CR, but expired within 2 months of the on-study date, then EFS was defined as the time from on-study until death regardless of cause. Otherwise, EFS was set at 2 months. The median event-free survival for the 44 overexpressing cases was 0.6 years (30%

23

event-free at 2 years) compared with all 7 non-overexpressing patients (100%) being event-free at 2 years (censored for the outcome; $P=0.004$; FIG. 16B). Disease-free survival (DFS) was defined only for patients achieving complete remission (CR) and was measured from the documented date of CR until date of relapse or death, regardless of cause, censoring for patients alive and in continuous CR. The median disease-free survival for 35 overexpressing cases (9 patients did not achieve complete remission) was 0.7 years (38% disease-free at 2 years) compared with all 7 non-overexpressing patients (100%) being disease-free at 2 years (censored for the outcome; $P=0.01$; FIG. 16C). OS, EFS and DFS were analyzed using the Kaplan-Meier method and the log-rank test was used to compare differences between survival curves.

The impact of other prognostic factors on OS and DFS was evaluated. Percentage of circulating blasts was significantly related to outcome (OS: $P=0.01$; DFS: $P=0.02$). Additionally, OS and DFS varied significantly across FLT3 genotype (OS: $P=0.009$; DFS: $P=0.002$). Because BAALC non-overexpressing patients were all censored for outcome, Cox proportional hazards models could not be used to adjust simultaneously for multiple independent prognostic factors. Therefore, restriction was used as a means of investigating the prognostic impact of BAALC expression, since confounding cannot occur if the prognostic factors do not vary across the two groups.

In an analysis restricted to patients with <77% circulating blasts (i.e. including all BAALC non-overexpressing patients), BAALC overexpressing patients had a significantly worse outcome than BAALC non-overexpressing patients [alive at 2 years (95% confidence intervals, CI): 53% (30%, 75%) vs 100%, $P=0.038$; disease-free at 2 years (95% CI): 56% (32%, 81%) vs 100%, $P=0.0497$]. In an analysis restricted to patients with the FLT3^{WT/WT} genotype, the 7 BAALC non-overexpressing patients remained alive and disease-free, whereas the 23 BAALC overexpressing patients showed a significantly inferior outcome [alive at 2 years (95% CI): 52% (32%, 73%), $P=0.01$; disease-free at 2 years (95% CI): 53% (29%, 77%), $P=0.03$]. Thus BAALC expression identifies high-risk patients within the prognostically favorable FLT3^{WT/WT} genotype group that fail to achieve long-term survival. Since FAB subtype differed significantly between BAALC overexpressing and BAALC non-overexpressing patients, an analysis restricted to patients with FAB subtypes M4/M5 was conducted. BAALC overexpressing patients showed a significantly inferior outcome [alive at 2 years: 31% (95% CI: 6%, 56%), $P=0.01$; disease-free at 2 years: 36% (95% CI: 8%, 65%), $P=0.02$] compared to BAALC non-overexpressing patients, indicating that the prognostic importance of BAALC expression is not accounted for by FAB subtype.

Example 6

BAALC Overexpression in CML Samples

Real-time PCR (TaqMan®, Applied Biosystems) was used to quantify the relative levels of BAALC transcripts in CML, using total RNA isolated from the cells in real-time PCR, as described in Example 2. Samples of leukocytes were obtained from eight patients with chronic phase CML. Samples of leukocytes were also obtained from eight patients with CML in blast crisis. Six out of the eight patients in blast crisis had

24

BAALC levels that were statistically significantly higher than BAALC levels in the eight patients in the chronic phase of CML.

Example 7

Generation of Polyclonal Antibodies Specific for BAALC

Polyclonal antibodies immunoreactive for BAALC were made by injection of peptide antigens into rabbits using standard techniques well known in the art. One peptide antigen, DAIEPRYYESWTRETEST (SEQ ID NO:35), was used to make the polyclonal antibody GN2214. Another peptide antigen, DSIQQMDRSRRITK (SEQ ID NO:36), was used to make the polyclonal antibody GN2216. Approximately 10 mg of each peptide was synthesized using standard methods. Each peptide was conjugated to either KLH or BSA (2-3 mg) and the conjugate was purified using gel filtration. The conjugated peptides were injected subcutaneously into rabbits. The initial injection (day 0) was in complete Freund's adjuvant and all subsequent immunizations (days 14, 28, 42, 56 and 70) were given in incomplete Freund's adjuvant. Sera was collected on days 49, 63 and 77. Antibodies were purified from the sera using standard methods.

Example 8

Histochemical Analysis of Prostate Cancer

Immunohistochemistry was performed on human prostate cancer tissue sections using the anti-human BAALC-specific antibody, GN2214, generated as described in Example 7 above. Formalin-fixed, paraffin-embedded tissue blocks were used as the tissue source. Control tissue was also available and processed as was the tumor tissue. Five-micron sections were from the paraffin-embedded tumor specimens and mounted on polylysine-coated slides. The sections were deparaffinized in xylene and hydrated through alcohols to saline. Slides were incubated with GN2214 at various dilutions, overnight at 4° C. and then subsequently washed in phosphate-buffered saline (PBS). The slides were then incubated with goat anti-rabbit antibody which was conjugated to fluorescein and also washed in PBS. The slides were visualized under a fluorescence microscope. More intense staining was observed in tissue samples from the majority of prostate cancers as compared to control, non-cancerous tissue.

Example 9

Generation of a Monoclonal Antibody Specific for BAALC Proteins

A monoclonal antibody is made using standard hybridoma techniques that are well known in the art. Briefly, the peptide RADAIEPRYYESWTRETESTWLTYT (SEQ ID NO:37) was injected into BALB/c mice with complete Freund's adjuvant. Two weeks later, a second immunization with the peptide in incomplete Freund's adjuvant is given. Five days before fusion, a third immunization with the peptide, absent adjuvant is given. Mice are sacrificed and the spleens are dissected. Spleen cells are dispersed and then fused to SP2/0 cells using 50% PEG. Fused cells are selected in medium containing HAT. Individual clones are expanded and supernatant is tested for antibodies reactive with BAALC protein.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 39

<210> SEQ ID NO 1
<211> LENGTH: 343
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

gagagggccc ggactagggg cggcgggcac cgcaggagct ccgcgcggct gcagcgcggg 60
cgggagcggg gacgcgatgt cgccgccgcc gcctccttgc gggccggggc tgcgcctccg 120
gggctgagcc gccgccagag ccgacagccg agcagccgct gggcgctccc gcggcgcagg 180
aggatgggct gcggcgggag cggggcggat gccatcgagc cccgctacta cgagagctgg 240
accggggaga cagaatccac ctggctcacc tacaccgact cggacgcgcc gccagcgcc 300
gccgcscgg acagcggccc cgaagcgggc ggctgcact cgg 343

<210> SEQ ID NO 2
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

cccattacc tcttgcttt gcacttgctt ggagagacaa cagtttaggg gctctgctgg 60
ttcaagaagg actgtgcagg tagcatggcc acacaccatg tacag 105

<210> SEQ ID NO 3
<211> LENGTH: 147
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

gttctggtgc ttaggagtgg acatctttgg gaccgagggt tattctgcct tcctaccatg 60
tcaccagagt tgtgctaata cacagagagc ttcaggggat gagatctgcc attcattgag 120
caccttctgt gcggcagaca gtgttag 147

<210> SEQ ID NO 4
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

ggtgccttga ggaacattac catctgactg ccctacagaa agttgggcat cccaaccatt 60
gatttaaaaa g 71

<210> SEQ ID NO 5
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

ttctggaggc tgagaagtcc aagatcaagg caccaacaga ttcagtgtct gatgaaggct 60
tggtctctgc ttcaaagatg gcacctcttg ctgtgttctc acatg 105

<210> SEQ ID NO 6
<211> LENGTH: 167
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 6

gcatgctgga agatggactg cctccaatg gtgtgccccg atctacagcc ccaggtggaa 60

tacccaaccc agagaagaag acgaactgtg agaccagtg cccaaatccc cagagcctca 120

gctcaggccc tctgaccag aacagaatg gccttcagac cacagag 167

<210> SEQ ID NO 7

<211> LENGTH: 90

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

gttctgctgc cttcctaattg tctgatcttc tatctgaagt tcaagttgaa aagcaacaac 60

tccttttggc actcgataca aactcccagg 90

<210> SEQ ID NO 8

<211> LENGTH: 2317

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

gctaaaagag atgctaagag aatgcctgca aaagaagtca ccattaatgt aacagatagc 60

atccaacaga tggacagaag tcgaagaatc acaaagaact gtgtcaacta gcagagagtc 120

caagcagaag ggcagatgga cttcttcagt gtccttcacg gcactggatc ccatcaaaga 180

accttgaaga agtggctgcc ccttgctgga cctgaattct actgagtccc tggcaagact 240

gtcttacctg gcagcaaact gctgcctgat ttgttgggac cttctgagcc ttctacttat 300

catgtaaattg tattggcaca gtgcttacat atgttaataa actgcaaattg tgcagttcag 360

tttgtctctt tgcaactcct gtaatacggg ctggtgtaaa agtagtgagt taaagctaca 420

ggtcagttta tgaacacaga aagtaggaat gcattttctg ggtgaaagag tcacacctta 480

gtgctataac tctcctgccc atgatagtgt attctgtttc aggcaagctt attctttcct 540

tctttcattt taaatattgt cattacaaat cttaccaggg tcacttaaaa gctggccttc 600

atccaactct aaaccacat attgaaaaaa tcaagggtaca ggaaaactcc ttgttatcct 660

tgtttcctta gcttggtatg agacagatcg gatccagttt cccatgcacc aaccactgc 720

ccatggcatg tctttgggag gtgtctgtga agcagtcata cctgctcctc atctgcctgg 780

aaagtccctc tattccagtg tccatgttgg cctccagtc ttaatgtcac catgcttgtg 840

gccaatgcat ccaaataagg ataccctca gggctcagct agacattgca attttgcata 900

gctttccagt tccctttgct tgtcttcttg actgtcttcc ctctctatcg gggtcacttg 960

caattgttaa tcaaagattg aactgcgt aggagaggga gatgatccag agacatgtgg 1020

cagcaggcat ggcttcccct tggcctctct gtacactgcc ccaggactgt cattttggca 1080

tctgcaaagg aatcacttta gaaagccagc acctggttga tgtgtattca tactgacatt 1140

agattgatgt gcactgcatt agaaatgagg tagctgacac agaaaaagga tgttttgata 1200

ggaataattt tctagtatgt cttgaaacat gttcatctgg aagtattttc ctccaaagta 1260

atgtagcatg atttttcaag gattgttaac atgcctggga ttgggaaaga taggactaaa 1320

gttgtgccaa actatatcaa taaattccat gtttagcaga aataggcagc ctattggtgt 1380

tatgtttatg taacatagtc cagagaactg acatgcaggt caaaagtcag atacgcaacc 1440

tccttatctg ctaactctgt tattcttcaa acacaacgtg ggtagtgtca tttttccttc 1500

-continued

cttccttcca	ttggcagatt	gtatatattat	tcacaaaaca	ttaaagtcc	atcctgtgcc	1560
aggtactatg	cagatgttga	gggatttggg	gtctggttag	tcgtgactat	ctatcctgaa	1620
tctaacagtg	acttcataac	taggagactg	aattagaccc	ttaaggtata	gtgtgtgttg	1680
caaatcactc	tgcaatggaa	acttttatat	tcagggtagg	tttgtgtctt	aaactaggtg	1740
ttctaataca	tgtacaagac	tttaccatac	acgcaactat	agtttttcta	aaccttcac	1800
attttgtgat	tctttgagaa	agggctttta	ggaactttat	gttctaaaaa	atgtttttta	1860
caataataag	ataaaagaaa	aacctgtgat	tcatatgtcc	ccactggcat	tactcagcag	1920
gagccccag	ctgccaaagg	ttggcagtga	tcttgcaagt	tcaagggctc	tttctccctg	1980
gggatgtgct	ttgtggcttc	tctttacagc	tttgtttctg	catcagttca	ctgctgcatg	2040
ttgtttggaa	tttatcacct	taagaaagtg	tctctgtttt	atatagaaac	actttctcac	2100
ttacagggga	gaaggaaatg	cagggcacat	gatctggccc	tccccagaac	aatctggatt	2160
tcacggagac	agcaaccaga	agttaaacca	tgtgactaaa	aatgcatctg	gctacttttt	2220
catgtatgta	tgagacagaa	actaatcctt	actatcctat	taggatacca	cttttcattg	2280
caaagtttgt	gtcaataaag	tcattaattt	taaacat			2317
<210> SEQ ID NO 9						
<211> LENGTH: 2826						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 9						
gagagggccc	ggacuagggg	cggcgggcac	cgcaggagcu	ccgcgcggcu	gcagcgcggg	60
ggggagcggg	gacgcgaugu	cgccgcgcgc	gccuccuugc	gggccggggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgaggg	180
aggaugggcu	gcggcgggag	ccgggcggau	gccaucgagc	cccgcuaqua	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gcccagcgcc	300
gccgcscggg	acagcgggcc	cgaagcgggc	ggccugcacu	cgggcaugcu	ggaagaugga	360
cugcccucca	auggugugcc	ccgaucuaca	gccccaggug	gaauaccaa	cccagagaag	420
aagacgaacu	gugagacca	gugcccaaau	ccccagagcc	ucagcucagg	cccucugacc	480
cagaaacaga	auggccuuca	gaccacagag	gcuaaaagag	augcuaagag	aaugccugca	540
aaagaaguca	ccauuaugu	aacagauagc	auccaacaga	uggacagaag	ucgaagaau	600
acaagaacu	gugucaacua	gcagagaguc	caagcagaag	ggcagaugga	cuucuucagu	660
guccuucacg	gcacuggauc	ccaucaaaga	accuugaaga	aguggcugcc	ccuugcugga	720
ccugaauucu	acugaguccc	uggcaagacu	gucuuaccug	gcagcaaacu	gcugccugau	780
uuguugggac	cuucugagcc	uucuacuau	cauguaaaug	uauuggcaca	gugcuuacau	840
auguaauaa	acugcaaaug	ugcaguucag	uuugucucu	ugcaacuccu	guaauacggg	900
cugguguaaa	aguagugagu	uaaagcuaca	ggucaguua	ugaaacagaa	aaguaggau	960
gcuuuuucug	ggugaaagag	ucacaccuua	gugcuauaac	ucuccugccc	augauagugu	1020
auucuguuuc	aggcaagcuu	auucuuuuccu	ucuuucauuu	uaauauuugu	cauuacaaa	1080
cuuaccaggu	ucacuuaaaa	gcuggcuuuc	auccaacucu	aaaccacau	auugaaaaa	1140
ucaagguaca	ggaaaacucc	uuguuauccu	uguuuccuua	gcuugguau	agacagaucg	1200
gauccaguuu	cccaugcacc	aaccacugc	ccauggcaug	ucuuugggag	gugucuguga	1260
agcagucua	ccugcuccuc	aucugccugg	aaaguccucc	uauuccagug	uccauguugg	1320

- continued

ccuccagucc	uuaaugucac	caugcuugug	gccaaugcau	ccaaauaagg	auaccccuca	1380
gggcucagcu	agacauugca	auuuugcaua	gcuuuccagu	ucccuuugcu	ugucuucuuug	1440
acugucuucc	cucucuaucg	gggucacuug	caauuuuaau	caaagauuga	acacugcgua	1500
ggagagggag	augauccaga	gacauguggc	agcaggcaug	gcuuccccuu	ggccucucug	1560
uacacugccc	caggacuguc	auuuuggcau	cugcaaagga	aucacuuuag	aaagccagca	1620
ccugguugau	guguauuau	acugacauua	gauugaugug	cacugcauua	gaaaugaggu	1680
agcugacaca	gaaaaaggau	guuuugauag	gaauauuuu	cuaguauguc	uugaaacaug	1740
uucaucugga	aguauuuucc	uccaaaguaa	uguagcauga	uuuuucaagg	auuguuaaca	1800
ugccugggau	ugggaaagau	aggacuaaag	uugugccaaa	cuauaucaau	aaaauccaug	1860
uuuagcagaa	auaggcagcc	uauugguguu	auguuuauu	aacauagucc	agagaacuga	1920
caugcagguc	aaaagucaga	uacgcaaccu	ccuuauucug	uaacucuguu	auucuucaaa	1980
cacaacgugg	guagugucau	uuuuccuucc	uuccuuccau	uggcagauug	uauauuuauu	2040
cacaaaacau	uaaaugucca	uccugugcca	gguacuaugc	agauguugag	ggauuugggg	2100
ucugguuagu	cgugacuau	uauccugaau	cuaacaguga	cuucauaacu	aggagacuga	2160
auuagacccu	uaagguauag	uguguguugc	aaaucacucu	gcaauggaaa	cuuuuauauu	2220
cagggauaggu	uugugucua	aacuaggugu	ucuaaucaau	guacaagacu	uuaccauaca	2280
cgcaacuaua	guuuuucuaa	accuucauca	uuuugugauu	cuuugagaaa	gggcuuuuag	2340
gaacuuuau	uucuaaaaaa	uguuuuuuac	aaauauaaga	uaaaagaaaa	accugugauu	2400
cauauugccc	cacuggcuuu	acucagcagg	agccccagc	ugccaaaggu	uggcagugau	2460
ccugcaaguu	caagggcucu	uucucccugg	ggaugugcuu	uguggcucuu	cuuuacagcu	2520
uuguuucugc	aucaguucac	ugcugcaugu	uguuuggaau	uuaucccuu	aagaaagugu	2580
cucuguuuua	uauagaaaca	cuuucucacu	uacaggggag	aaggaaaugc	agggcacaug	2640
aucugggccu	ccccagaaca	aucuggauuu	cacggagaca	gcaaccagaa	guuaaaccu	2700
gugacuaaaa	augcaucugg	cuacuuiuuc	auguauguau	gagacagaaa	cuaauccuua	2760
cuauccuauu	aggauaccac	uuuucuuugc	aaaguuuugug	ucaauaaaagu	cauuauuuuu	2820
aaacau						2826
<210> SEQ ID NO 10						
<211> LENGTH: 2660						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 10						
gagagggccc	ggacuagggg	cggcgggcac	cgcaggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgcgcggccc	gccuccuugc	gggcgggggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgaggg	180
aggaugggcu	gcggcgggag	ccgggaggau	gccaucgagc	cccgcuaacu	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gccagcgccc	300
gccgcscggg	acagcggccc	cgaagcgggc	ggccugcacu	cgggcuaaaa	gagaugcuua	360
gagaaugccu	gcaaaagaag	ucaccauuaa	uguuacagau	agcauccaac	agauggacag	420
aagucgaaga	aucacaaaaga	acugugucua	cuagcagaga	guccaagcag	aagggcagau	480
ggacuucuu	aguguccuuc	acggcacugg	aucccaucaa	agaaccuuga	agaaguggcu	540

- continued

gccccuugcu	ggaccugaau	ucuacugagu	cccuggcaag	acugucuuac	cuggcagcaa	600
acugcugccu	gauuuguugg	gaccuucuga	gccuucucu	uaucauguaa	auguauuggc	660
acagugcuua	cauauuguua	uaaacugcaa	augugcaguu	caguuuugucu	cuuugcaacu	720
ccuguaauac	ggucuggugu	aaaaguagug	aguuaaagcu	acaggucagu	uuaugaaaca	780
gaaaaguagg	aaugcauuuu	cugggugaaa	gagucacacc	uuagugcuau	aacucuccug	840
cccagauag	uguauucugu	uucaggcaag	cuuauucuuu	ccuucuuuca	uuuuaaaaua	900
ugucauuaca	aaucuuacca	gguucacuaa	aaagcugggc	uucauccaac	ucuaaaccca	960
cauauugaaa	aaaucaaggu	acaggaaaac	uccuuguuau	ccuuguuucc	uuagcuuggu	1020
augagacaga	ucggauccag	uuucccaugc	accaaccac	ugcccauggc	augucuugg	1080
gaggugucug	ugaagcaguc	auaccugcuc	cucaucugcc	uggaaagucc	uccuauucca	1140
guguccaugu	uggccuccag	uccuuuauug	caccaugcuu	guggccaaug	cauccaaaaua	1200
aggauacccc	ucagggcuca	gcuagacauu	gcauuuuugc	auagcuuucc	aguucccuuu	1260
gcuugucuuc	uugacugucu	ucccucucua	ucggggucac	uugcaauugu	uaaucaaaga	1320
uugaacacug	cguaggagag	ggagaugauc	cagagacaug	uggcagcagg	cauggcuucc	1380
ccuuggccuc	ucuguacacu	gccccaggac	ugucauuuug	gcaucugcaa	aggaauccacu	1440
uuagaaagcc	agcaccuggu	ugauguguau	ucauacugac	auuagauuga	ugugcacugc	1500
auuagaaaug	agguagcuga	cacagaaaaa	ggauuuuug	auaggaauaa	uuuucuagua	1560
ugucuugaaa	cauguuauu	uggaaguauu	uuccuccaaa	guaauguagc	augauuuuuc	1620
aaggauuguu	aacaugccug	ggauugggaa	agauaggacu	aaaguugugc	caaacuauau	1680
caauaaaauu	cauguuuagc	agaaauaggc	agccuauugg	uguuauuuuu	auguaacaua	1740
guccagagaa	cugacaugca	ggucaaaagu	cagauacgca	accuccuuau	cugcuaacuc	1800
uguuauucuu	caaacacaac	guggguagug	ucauuuuucc	uuccuuccuu	ccauuggcag	1860
auuguauauu	uauucacaaa	acauuaaau	uccauccugu	gccagguacu	augcagaugu	1920
ugagggauuu	ggggucuggu	uagucgugac	uaucauuccu	gaaucauaca	gugacuucuu	1980
aacuaggaga	cugaauuaga	cccuuaaggu	auagugugug	uugcaauuca	cucugcaaug	2040
gaaacuuuuu	uauucagggu	agguuugugu	cuuaaacuag	guguucuaau	caauguacaa	2100
gacuuuacca	uacacgcaac	uauaguuuuu	cuaaacuucc	aucauuuugu	gauucuuuga	2160
gaaagggcuu	uuaggaacuu	uauguucuaa	aaaauuuuuu	uaacaauaa	aagauaaaag	2220
aaaaaccugu	gauucauau	uccccacug	cauucucag	caggagcccc	cagcugccaa	2280
agguuggcag	ugaucugca	aguucaaggg	cucuuucucc	cuggggauug	gcuuuguggc	2340
uucucuuuac	agcuuuguuu	cugcaucagu	ucacugcugc	auguuguuuu	gaauuuauca	2400
ccuaaagaaa	gugucucugu	uuuauauaga	aacacuuccu	cacuucacag	ggagaaggaa	2460
augcagggca	caugaucugg	cccucuccag	aacaaucugg	auuucacgga	gacagcaacc	2520
agaaguuaaa	ccaugugacu	aaaaaugcau	cuggcuacuu	uuucauguau	guaugagaca	2580
gaaacuaauc	cuuacuaucc	uauuaggaua	ccacuuccu	uugcaaguu	ugugucaaua	2640
aagucauuua	uuuuuacau					2660

<210> SEQ ID NO 11
<211> LENGTH: 2931
<212> TYPE: RNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

- continued

gagagggccc	ggacuagggg	cggcgggcac	cgcaggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgccgccgcc	gccuccuugc	gggccggggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgcagg	180
aggaugggcu	gcggcgggag	ccgggcgga	gccaucgagc	cccgcuaqua	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gccagcgcc	300
gccgcscgg	acagcggccc	cgaagcgggc	ggccugcacu	cgguucugga	ggcugagaag	360
uccaagauca	aggcaccaac	agauucagug	ucugaugaag	gcuuguucuc	ugcuucaaag	420
auggcaccuc	uugcuguguu	cucacauggc	augcuggaag	auggacugcc	cuccauggu	480
gugccccgau	cuacagcccc	agguggaaua	cccaaccag	agaagaagac	gaacugugag	540
acccagugcc	caaaucacca	gagccucagc	ucaggcccuc	ugaccagaa	acagauggc	600
cuucagacca	cagaggcuaa	aagagaugcu	aagagaugc	cugcaaaaga	agucaccau	660
aauguaacag	auagcaucca	acagauggac	agaagucgaa	gaaucaaaa	gaacuguguc	720
aacuagcaga	gaguccaagc	agaagggcag	auggacuucu	ucaguguccu	ucacggcacu	780
ggaucacca	aaagaaccuu	gaagaagugg	cugcccuug	cuggaccuga	auucuacuga	840
guccugggca	agacugucuu	accuggcagc	aaacugcugc	cugauuuguu	gggaccuucu	900
gagccuucua	cuuaucaugu	aaauguauug	gcacagugcu	uacauauguu	aaauaacugc	960
aaaugugcag	uucaguuuu	cucuuggcaa	cuccuguaau	acggucuggu	guaaaaguag	1020
ugaguuaaag	cuacagguca	guuuauaaa	cagaaaagua	ggaugcauu	uucuggguga	1080
aagagucaca	ccuuagugcu	auaacucucc	ugcccaugau	aguguaucuu	guuucaggca	1140
agcuuauucu	uuccuucuuu	cauuuuuuu	auugucauu	caauucuuac	cagguucacu	1200
uaaaagcugg	cuuucacucca	acucuaaacc	cacauauuga	aaaaaucaag	guacaggaaa	1260
acuccuuguu	auccuuguuu	ccuuagcuug	guauagagaca	gaucggaucc	aguuuccau	1320
gcaccaaccc	acugcccaug	gcaugucuuu	gggagguguc	ugugaagcag	ucauaccugc	1380
uccucaucug	ccuggaaagu	ccuccuauuc	caguguccau	guuggccucc	aguccuaau	1440
gucaccaugc	uuguggccaa	ugcauccaaa	uaaggauacc	ccucagggcu	cagcuagaca	1500
uugcauuuu	gcuaugcuuu	ccaguucccu	uugcuugucu	ucuugacugu	cuuccucuc	1560
uacggggguc	acuugcaauu	guuaaucaaa	gauugaacac	ugcguaaggag	agggagauga	1620
uccagagaca	uguggcagca	ggcauggcuu	ccccuuggcc	ucucuguaca	cugccccagg	1680
acugucauuu	uggcaucugc	aaaggaauc	cuuagaaaag	ccagcaccug	guugaugugu	1740
auucauacug	acauuagauu	gaugugcacu	gcauuagaaa	ugagguagcu	gacacagaaa	1800
aaggauuuu	ugauaggaau	aaauuucua	uauugcuuga	aacauuguca	ucuggaagua	1860
uuuuccucca	aaguaaugua	gcaugauuuu	ucaaggauug	uaaacaugcc	ugggauuggg	1920
aaagauagga	cuaaaguugu	gccaaacuau	aucaauaaa	uccauguuua	gcagaaaau	1980
gcagccuauu	gguguuauu	uuauuaaca	uaguccagag	aacugacaug	caggucaaaa	2040
gucagauacg	caaccuccuu	aucugcuaac	ucuguuauuc	uucaaacaca	acguggguag	2100
ugucauuuuu	ccuuccuucc	uuccauuggc	agauuguaua	uuuauucaca	aaacauuaaa	2160
uguccauccu	gugccaggua	cuaugcagau	guugagggau	uuggggucug	guuagucgug	2220
acuaucuauc	cugaaucuua	cagugacuuc	auaacuagga	gacugaauua	gaccuuuaag	2280
guauagugug	uguugcaauu	cacucugcaa	uggaaacuuu	uauauucagg	guagguuugu	2340

-continued

gucuuaaacu	agguguucua	aucaauguac	aagacuuuac	cauacacgca	acuaauaguuu	2400
uucuaaaccu	ucaucauuuu	gugauucuuu	gagaaagggc	uuuuaggaac	uuuanguuca	2460
aaaaauguuu	uuaacaauaa	uaagauaaaa	gaaaaaccug	ugauucauau	gucccccacug	2520
gcuuuacuca	gcaggagccc	ccagcugcca	aagguuggca	gugauccugc	aaguucaagg	2580
gcucuucuc	ccuggggaug	ugcuuugugg	cuucucuuaa	cagcuuuguu	ucugcaucag	2640
uucacugcug	cauguuguuu	ggaauuuau	accuuuagaa	agugucucug	uuuuauauag	2700
aaacacuuuc	ucacuucacag	gggagaagga	aaugcagggc	acaugaucug	gcccucccca	2760
gaacaaucug	gauuucacgg	agacagcaac	cagaaguuaa	accaugugac	uaaaaaugca	2820
ucuggcuacu	uuuucaugua	uguaugagac	agaaacuaau	ccuacuauc	cuauuaggau	2880
accacuuuuc	auugcaaagu	uugugucaau	aaagucauaa	auuuuaaaca	u	2931
<210> SEQ ID NO 12						
<211> LENGTH: 3003						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 12						
gagagggccc	ggacuagggg	cggcggggcac	cgcaggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgccgccgcc	gccuccuugc	gggccggggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgcagg	180
aggaugggcu	gcggcgggag	ccgggcgga	gccaucgagc	cccgcuaa	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gcccagcgcc	300
gccgcscgg	acagcggccc	cgaagcgggc	ggccugcacu	cggggugccu	ugaggaacau	360
uaccaucuga	cugcccuaca	gaaaguuggg	caucccaacc	auugauuuaa	aaaguucugg	420
aggcugagaa	guccaagauc	aaggcaccaa	cagauucagu	gucugaugaa	ggcuuguucu	480
cugcuucaa	gauggcaccu	cuugcugugu	ucucacaugg	caugcuggaa	gauggacugc	540
ccuccaaugg	ugugccccga	ucuacagccc	cagguggaau	acccaaccca	gagaagaaga	600
cgaacuguga	gaccagugc	ccaaaucucc	agagccucag	cucaggcccu	cugaccaga	660
aacagaau	ccuucagacc	acagaggcua	aaagagaugc	uaagagaau	ccugcaaaag	720
aagucaccau	uaauguaaca	gauagcaucc	aacagaugga	cagaagucga	agaaucaaa	780
agaacugugu	caacuagcag	agaguccaag	cagaagggca	gauggacuuc	uucagugucc	840
uucacggcac	uggaucccau	caaagaaccu	ugaagaagug	gcugccccc	gcuggaccug	900
aauuuacug	agucccuggc	aagacugucu	uaccuggcag	caaacugcug	ccugauuugu	960
ugggaccuuc	ugagccuucu	acuuaucaug	uaaauguauu	ggcacagugc	uuacauaugu	1020
uaauaaacug	caaaugugca	guucaguuu	ucucuugca	acuccuguaa	uacggucugg	1080
uguaaaagua	gugaguuaaa	gcuacagguc	aguuuuagaa	acagaaaagu	aggaaugcau	1140
uuucugggug	aaagagucac	accuuagugc	uauaacucuc	cugcccauga	uaguguauuc	1200
uguuucaggc	aagcuuauuc	uuuccuucuu	ucauuuuaaa	uauugucauu	acaaaucuaa	1260
ccagguucac	uuaaaagcug	gcuuucaucc	aacucuaaac	ccacauauug	aaaaaucaa	1320
gguacaggaa	aacuccuugu	uauccuuguu	uccuuagcuu	gguaugagac	agaucggauc	1380
caguuuccca	ugcaccaacc	cacugccc	ggcaugucuu	ugggaggugu	cugugaagca	1440
gucauaccug	cuccucaucu	gccuggaaag	uccuccuauu	ccagugucca	uguuggccuc	1500
caguccuuaa	ugucaccaug	cuuguggcca	augcauccaa	auaaggauac	cccucagggc	1560

- continued

ucagcuagac	auugcaauuu	ugcauagcuu	uccaguuccc	uuugcuuguc	uucuugacug	1620
ucuucccucu	cuaucggggu	cacuugcaau	uguuaaucaa	agauugaaca	cugcguagga	1680
gagggagaug	auccagagac	auguggcagc	aggcauggcu	uccccuuggc	cucucuguac	1740
acugccccag	gacugucauu	uuggcaucug	caaaggaauc	acuuuagaaa	gccagcaccu	1800
gguugaugug	uauucauacu	gacauuagau	ugaugugcac	ugcauuagaa	augagguagc	1860
ugacacagaa	aaaggauuu	uugauaggaa	uaauuuucua	guaugucuug	aaacauguuc	1920
aucuggaagu	auuuuccucc	aaaguaaugu	agcaugauuu	uucaaggauu	guuaacaugc	1980
cugggauugg	gaaagauagg	acuaaaguug	ugccaaacua	uaucaauaaa	uuccauguuu	2040
agcagaaaua	ggcagccuau	ugguguuau	uuuauguaac	auaguccaga	gaacugacau	2100
gcaggucaaa	agucagauac	gcaaccuccu	uaucugcuaa	cucuguuauu	cuucaaacac	2160
aacgugggua	gugucauuuu	uccuuccuuc	cuuccauugg	cagauuguau	auuuauucac	2220
aaaacauuaa	auguccaucc	ugugccaggu	acuaugcaga	uguugaggga	uuuggggucu	2280
gguuagucgu	gacuaucua	ccugaaucua	acagugacuu	cauaacuagg	agacugaauu	2340
agacccuuaa	gguaauagug	guguugcaaa	ucacucugca	auggaaacuu	uuauauucag	2400
gguaagguug	ugucuuaaac	uagguguucu	aaucaaugua	caagacuuaa	ccauacacgc	2460
aacuaauagu	uuucuaaacc	uucaucuuu	ugugauucuu	ugagaaaggg	cuuuuaggaa	2520
cuuuauguuc	uaaaaaaugu	uuuuuacaau	aaauagauaa	aagaaaaacc	ugugauucau	2580
auguccccac	uggcauuacu	cagcaggagc	ccccagcugc	caaagguugg	cagugauccu	2640
gcaaguucua	gggcucuuuc	ucccugggga	ugugcuuugu	ggcuucucuu	uacagcuuug	2700
uuucugcauc	aguucacugc	ugcauguugu	uuggaauuua	ucaccuuaag	aaagugucuc	2760
uguuuuauau	agaaacacuu	ucucacuuac	aggggagaag	gaaaugcagg	gcacaugauc	2820
uggcccccac	cagaacaauc	uggauuucac	ggagacagca	accagaaguu	aaaccaugug	2880
acuaaaaaug	caucuggcua	cuuuuucaug	uauguaugag	acagaaacua	auccuuacua	2940
uccuauuagg	auaccacuuu	ucauugcaaa	guuuguguca	auaaagucan	uaauuuuaaa	3000
cau						3003
<210> SEQ ID NO 13						
<211> LENGTH: 3022						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 13						
gagagggccc	ggacuagggg	cggcgggcac	cgagggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgcgcggcc	gccuccuugc	gggcccgggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgaggg	180
aggaugggcu	gcggcgggag	ccgggaggau	gccaucgagc	cccgcuaacua	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gcccagcgcc	300
gccgcscggg	acagcgggcc	cgaagcgggc	ggccugcacu	cgguucugga	ggcugagaag	360
uccaagauca	aggcaccaac	agauucagug	ucugaugaag	gcuuguucuc	ugcuucaaag	420
auggcaccuc	uugcuguguu	cucacauggc	augcuggaag	auggacugcc	cuccauggu	480
gugccccgau	cuacagcccc	agguggaaua	ccaaccccag	agaagaagac	gaacugugag	540
acccagugcc	caauucccca	gagccucagc	ucaggcccuc	ugaccagaaa	acagaugggc	600

- continued

cuucagacca	cagagguucu	gcugccuucc	uaaugucuga	ucuucuaucu	gaaguucaag	660
uugaaaagca	acaacuccuu	uuggcacucg	auacaaacuc	ccagggcuaa	aagagaugcu	720
aagagaaugc	cugcaaaaaga	agucaccauu	aauguaacag	auagcaucca	acagauggac	780
agaagucgaa	gaaucacaaa	gaacuguguc	aacuagcaga	gaguccaagc	agaagggcag	840
auggacuucu	ucaguguccu	ucacggcacu	ggaucccauc	aaagaaccuu	gaagaagugg	900
cugccccuug	cuggaccuga	auucuacuga	gucccuuggca	agacugucuu	accuggcagc	960
aaacugcugc	cugauuuguu	gggaccuucu	gagccuucua	cuaaucaugu	aaauguauug	1020
gcacagugcu	uacauauguu	aauaaacugc	aaaugugcag	uucaguuuugu	cucuugcaa	1080
cuccuguaau	acggucuggu	guaaaaguag	ugaguuaaag	cuacagguca	guuuau meta	1140
cagaaaagua	ggaaugcauu	uucuggguga	aagagucaca	ccuagugcu	auaacucucc	1200
ugcccAugau	aguguauucu	guuucaggca	agcuuauucu	uuccuucuuu	cauuuu meta	1260
auugucauua	caaaucuuac	cagguucacu	uaaaagcugg	cuuucaucca	acucuaaacc	1320
cacauauuga	aaaaaucaag	guacaggaaa	acuccuuguu	auccuuguuu	ccuagcuug	1380
guaugagaca	gaucggaucc	aguuucccau	gcaccaaccc	acugcccaug	gcaugucuuu	1440
gggagguguc	ugugaagcag	ucauaccugc	uccucaucug	ccuggaaagu	ccuccuauuc	1500
caguguccau	guuggccucc	aguccuuaau	gucaccaugc	uuguggccaa	ugcauccaaa	1560
uaaggauacc	ccucagggcu	cagcuagaca	uugcauuuuu	gcauagcuuu	ccaguucccu	1620
uugcuugucu	ucuugacugu	cuucccucuc	uaucgggguc	acuugcaauu	guuaau meta	1680
gauugaacac	ugcguaggag	agggagauga	uccagagaca	uguggcagca	ggcauggcuu	1740
ccccuuggcc	ucucuguaca	cugccccagg	acugucauuu	uggcaucugc	aaaggaauca	1800
cuuuagaaag	ccagcaccug	guugaugugu	auucauacug	acauuagauu	gaugugcacu	1860
gcauuagaaa	ugagguagcu	gacacagaaa	aaggauuguu	ugauaggauu	aauuuucuag	1920
uauugcuuga	aacauguuca	ucuggaagua	uuuuccucca	aaguaaugua	gcaugauuuu	1980
ucaaggauug	uuuacaugcc	ugggauuggg	aaagauagga	cuaaaguugu	gccaaacuau	2040
aucaau meta	uccauguuua	gcagaaauag	gcagccuauu	gguguuaugu	uuau meta	2100
uaguccagag	aacugacaug	cagguc meta	gucagauacg	caaccuccuu	aucugcuaac	2160
ucuguuauuc	uu meta	acguggguag	ugucauuuuu	ccuuccuucc	uuccauuggc	2220
agauuguaua	uuuauucaca	aaacau meta	uguccauccu	gugccaggua	cuaugcagau	2280
guugagggau	uuggggucug	guuagucgug	acuaucuauc	cugaaucuaa	cagugacuuc	2340
auaacuagga	gacugaauua	gaccuu meta	guauagugug	uguug meta	cacucugcaa	2400
uggaaacuui	uauauucagg	guagguuuugu	gucuu meta	agguguucua	aucaauguac	2460
aagacuuii	cauacacgca	acuauaguui	uucuaaaccu	ucaucauuui	gugauucuii	2520
gagaaagggc	uuuuaggaac	uuuauuguuc	aaaaaauguu	uuua meta	auaagau meta	2580
ag meta	gugauucaua	uguccccacu	ggcauuacuc	agcaggagcc	cccagcugcc	2640
aaagguuggc	agugauccug	caaguucaag	ggcucuiiuc	cccuggggau	gugcuuugug	2700
gcuucucuii	acagcuuugu	uucugcauca	guucacugcu	gcauguuguu	uggaauiiua	2760
caccuii meta	aagugucucu	guuuuau meta	gaaacacuii	cucacuiiua	ggggagaa	2820
aaaugcaggg	cacau meta	ggccuc meta	aga meta	ggauuucacg	gagacagcaa	2880
ccagaaguua	aaccauguga	cua meta	aucuggcuac	uuuu meta	auguaugaga	2940
cagaaacuua	uccuacuau	ccuauuagga	uaccacuii	cauug meta	uuugugucua	3000

- continued

uaaagucauu aaauuuuaaac au	3022
<210> SEQ ID NO 14	
<211> LENGTH: 2932	
<212> TYPE: RNA	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 14	
gagagggccc ggacuagggg cggcgggcac cgcaggagcu ccgcgcggcu gcagcgcggg	60
cgggagcggg gacgcgaugu cgccgccgcc gccuccuugc gggccggggc ugcgccuccg	120
gggcugagcc gccgccagag ccgacagccg agcagccgcu gggcgcuccc gcggcgcagg	180
aggaugggcu gcggcgggag ccgggcggau gccaucgagc cccgcuaqua cgagagcugg	240
acccgggaga cagaauccac cugggcucacc uacaccgacu cggacgcgcc gccagcgcc	300
gccgcscgg acagcggccc cgaagcgggc ggccugcacu cggcccauaa cccucuugcc	360
uuugcacuug ccuggagaga caacaguuaa ggggcucugc ugguucaaga aggacugugc	420
agguagcaug gccacacacc auguacaggc augcuggaag auggacugcc cuccaauggu	480
gugccccgau cuacagcccc agguggaaua cccaaccag agaagaagac gaacugugag	540
acccagugcc caaaucCCA gagccucagc ucaggcccuc ugaccagaa acagauggc	600
cuucagacca cagaggcuaa aagagaugcu aagagaauGC cugcaaaaga agucaccau	660
aauguaacag auagcaucca acagauggac agaagucgaa gaucacaaa gaacuguguc	720
aacuagcaga gaguccaagc agaagggcag auggacuucu ucaguguccu ucacggcacu	780
ggaucCauc aaagaaccuu gaagaagugg cugccccuug cuggaccuga auucuacuga	840
guccugggca agacugucu accuggcagc aaacugcugc cugauuuguu gggaccuucu	900
gagccuucua cuuaucaugu aaauguauug gcacagugcu uacauauguu aaaaaacugc	960
aaaugugcag uucaguuguu cucuuugcaa cuccuguaau acggucuggu guaaaaguag	1020
ugaguuaaag cuacagguca guuuauGaaa cagaaaagua ggaaugcau uucuggguga	1080
aagagucaca ccuagugcu auaacucucc ugcccaugau aguguauucu guuucaggca	1140
agcuuauucu uuccuucuuu cauuuuaaa auugucaua caaaucuuac cagguucacu	1200
uaaaagcugg cuuucacca acucuaaacc cacauauuga aaaaaucaag guacaggaaa	1260
acuccuuguu auccuuguuu ccuagcuug guaugagaca gaucggaucc aguucccau	1320
gcaccaaccc acugcccaug gcaugucuuu gggagguguc ugugaagcag ucauaccugc	1380
uccucaucug ccuggaaagu ccuccuauuc caguguccau guuggccucc aguccuaau	1440
gucaccaugc uuguggccaa ugcauccaaa uaaggauacc ccucagggcu cagcuagaca	1500
uugcauuuu gcauagcuu ccaguucccu uugcuugcu ucuugacugu cuucccucuc	1560
uacggggguc acuugcaau guuaaucaaa gauugaacac ugcguaggag agggagauga	1620
uccagagaca uguggcagca ggcauggcuu ccccuuggcc ucucuguaca cugccccagg	1680
acugucauuu uggaucugc aaaggaauc cuuagaaag ccagcaccug guugaugugu	1740
auucauacug acauuagau gaugugcacu gcauuagaaa ugagguagcu gacacagaaa	1800
aaggauuuu ugauaggaau aauuuucuag uaugucuuga aacauguuca ucuggaagua	1860
uuuuccucca aaguaaugua gcaugauuuu ucaaggauug uuaacaugcc ugggauuggg	1920
aaagauagga cuaaaguugu gccaaacuau aucaauaaau uccauguua gcagaaauag	1980
gcagccuauu gguguuauGu uuauguaaca uaguccagag aacugacaug caggucaaaa	2040

- continued

gucagauacg	caaccuccuu	aucugcuaac	ucuguuauuc	uucaaacaca	acguggguag	2100
ugucauuuuu	ccuuccuucc	uuccauuggc	agauuguaua	uuuauucaca	aaacauuaaa	2160
uguccauccu	gugccaggua	cuaugcagau	guugagggau	uuggggucug	guuagucgug	2220
acuaucuauc	cugaaucuaa	cagugacuuc	auaacuagga	gacugaauua	gacccuaaag	2280
guauagugug	uguugcaaa	cacucugcaa	uggaaacuuu	uauauucagg	guagguuugu	2340
gucuuaaaacu	agguguucua	aucaauguac	aagacuuuac	cauacacgca	acuauaguuu	2400
uucuaaaccu	ucaucauuuu	gugauucuuu	gagaaagggc	uuuaggaac	uuuanguucu	2460
aaaaaanguu	uuuaacaa	auaagauaaa	agaaaaaccu	gugauucaua	uguccccacu	2520
ggcauuacuc	agcaggagcc	cccagcugcc	aaagguuggc	agugauccug	caaguucaag	2580
ggcucuuucu	cccuggggau	gugcuuugug	gcuucucuuu	acagcuuugu	uucugcauca	2640
guucacugcu	gcauguuguu	uggaauuuau	caccuuuaga	aagugucucu	guuuuuauua	2700
gaaacacuuu	cucacuuaca	ggggagaagg	aaaugcaggg	cacaugaucu	ggcccucccc	2760
agaacaaucu	ggauuucacg	gagacagcaa	ccagaaguua	aaccauguga	cuaaaaaugc	2820
aucuggcuac	uuuuucaugu	auguaugaga	cagaaacuaa	uccuuacuau	ccuauuagga	2880
uaccacuuuu	caugcaaaag	uuugugucaa	uaaagucauu	aauuuuaaac	au	2932
<210> SEQ ID NO 15						
<211> LENGTH: 3037						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 15						
gagaggggcc	ggacuagggg	cggcgggcac	cgagggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgcgcgcgcc	gccuccuugc	gggcgcgggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgaggg	180
aggaugggcu	gcggcgggag	ccgggcggau	gccaucgagc	cccgcuaqua	cgagagcugg	240
acccgggaga	cagaauccac	cugggcucac	uacaccgacu	cggacgcgcc	gcccagcgcc	300
gccgcscggg	acagcgggcc	cgaagcgggc	ggccugcacu	cggcccauuu	cccucuuagg	360
uuugcacuug	ccuggagaga	caacaguuuu	ggggcucugc	ugguucaga	aggacugugc	420
agguagcaug	gccacacacc	auguacagu	cuggagggcu	agaaguccaa	gaucagggca	480
ccaacagauu	cagugucuga	ugaaggcuug	uucucugcuu	caaagauggc	accucuuugc	540
guguucucac	auggcaugcu	ggaagaugga	cugcccucca	auggugugcc	ccgaucuaqa	600
gcccagggug	gaauacccaa	cccagagaag	aagacgaacu	gugagacca	gugcccaau	660
cccagagcc	ucagcucagg	cccucugacc	cagaaacaga	auggcuuca	gaccacagag	720
gcuaaaagag	augcuaagag	aaugccugca	aaagaaguca	ccauuaaugu	aacagauagc	780
auccaacaga	uggacagaag	ucgaagaauc	acaaagaacu	gugucaacua	gcagagaguc	840
caagcagaag	ggcagaugga	cuucuucagu	guccuucacg	gcacuggauc	ccaucaaga	900
accuugaaga	aguggcugcc	ccuugcugga	ccugaauucu	acugaguccc	uggcaagacu	960
gucuuaccug	gcagcaaacu	gcugccugau	uuguugggac	cuucugagcc	uucuaquuu	1020
cauguaaaug	uauuggcaca	gugcuuacau	auguuuuuu	acugcaaaug	ugcaguucag	1080
uuugucucuu	ugcaacuccu	guaauacggg	cugguguaaa	aguagugagu	uaaagcuaca	1140
ggucaguuuu	ugaaacagaa	aaguaggau	gcuuuuucug	ggugaaagag	ucacaccua	1200
gugcuuaaac	ucuccugccc	augauagugu	auucuguuuc	aggcaagcuu	auucuuuccu	1260

- continued

ucuuucauuu	uaaaauuugu	cauuacaaau	cuuaccaggu	ucacuuaaaa	gcuggcuuuc	1320
auccaacucu	aaaccacau	auugaaaaaa	ucaagguaca	ggaaaacucc	uuguuauccu	1380
uguuuccuua	gcuugguau	agacagaucg	gauccaguuu	cccaugcacc	aaccacugc	1440
ccauggcaug	ucuuugggag	gugucuguga	agcagucaua	ccugcuccuc	aucugccugg	1500
aaaguccucc	uauuccagug	uccauguugg	ccuccagucc	uuaaugucac	caugcuugug	1560
gccaaugcau	ccaaauaagg	auaccccuca	gggcucagcu	agacauugca	auuuugcaua	1620
gcuuuccagu	ucccuuugcu	ugucuucuug	acugucuucc	cucucuaucg	gggucacuug	1680
caauuguuaa	ucaaagauug	aacacugcgu	aggagaggga	gaugauccag	agacaugugg	1740
cagcaggcau	ggcuuccccu	uggccucucu	guacacugcc	ccaggacugu	cauuuuggca	1800
ucugcaaagg	aaucacuuua	gaaagccagc	accugguuga	uguguauuca	uacugacauu	1860
agauugaugu	gcacugcauu	agaaaugagg	uagcugacac	agaaaaagga	uguuuugaua	1920
ggaaauauuu	ucuaguaugu	cuugaaacau	guucaucugg	aaguauuuuc	cuccaaagua	1980
auguagcaug	auuuuucag	gauuguaac	augccuggga	uugggaaaga	uaggacuaaa	2040
guugugccaa	acuauaucaa	uaaaauccau	guuagcaga	aaauaggcagc	cuauuggugu	2100
uauguuuuug	uaacauaguc	cagagaacug	acaugcaggu	caaagucag	auacgcaacc	2160
uccuuauucug	cuaacucugu	uauucuucua	acacaacgug	gguaguguca	uuuuuccuuc	2220
cuuccuucca	uuggcagauu	guauauuuau	ucacaaaaca	uuaaaugucc	auccugugcc	2280
agguacuaug	cagauguuga	gggauuuggg	gucugguuag	ucgugacua	cuauccugaa	2340
ucuaacagug	acuucauaac	uaggagacug	aaauagaccc	uuaagguaua	guguguguug	2400
caauucacuc	ugcaauggaa	acuuuuauau	ucaggguaagg	uuugugucuu	aaacuaggug	2460
uucuaaucaa	uguacaagac	uuuaccuac	acgcaacuau	aguuuuucua	aaccuucauc	2520
auuuugugau	ucuuugagaa	agggcuuuua	ggaacuuuau	guucuaaaaa	auguuuuuaa	2580
caauaauaag	auaaaagaaa	aaccugugau	ucauauugucc	ccacuggcau	uacucagcag	2640
gagccccag	cugccaaagg	uuggcaguga	uccugcaagu	ucaagggcuc	uuucucccug	2700
gggaugugcu	uuguggcuuc	ucuuuacagc	uuuguuucug	caucaguuca	cugcugcaug	2760
uuguuuggaa	uuuauaccu	uaagaaagug	ucucuguuuu	auauagaaac	acuuucucac	2820
uuacagggga	gaaggaaaug	cagggcacau	gaucuggccc	uccccagaac	aaucuggauu	2880
ucacggagac	agcaaccaga	aguuaaacca	ugugacuaaa	aaugcaucug	gcuacuuuuu	2940
cauguaugua	ugagacagaa	acuaauccuu	acuauccuau	uaggauacca	cuuuucauug	3000
caaaguuuugu	gucaauaaaag	ucauuauuuu	uaaacau			3037
<210> SEQ ID NO 16						
<211> LENGTH: 3079						
<212> TYPE: RNA						
<213> ORGANISM: Homo sapiens						
<400> SEQUENCE: 16						
gagagggccc	ggacuagggg	cggcgggcac	cgcaggagcu	ccgcgcggcu	gcagcgcggg	60
cgggagcggg	gacgcgaugu	cgcgcgcgcc	gccuccuugc	gggcgggggc	ugcgccuccg	120
gggcugagcc	gccgccagag	ccgacagccg	agcagccgcu	gggcgcuccc	gcggcgcagg	180
aggaugggcu	gcggcgggag	ccgggcggau	gccaucgagc	cccgcuaqua	cgagagcugg	240
acccgggaga	cagaauccac	cuggcucacc	uacaccgacu	cggacgcgcc	gcccagcgcc	300

- continued

gccgcscggg	acagcggccc	cgaagcgggc	ggccugcacu	cgccccauua	cccucuugcc	360
uuugcacuug	ccuggagaga	caacaguuaa	ggggcucugc	ugguucaaga	aggacugugc	420
agguagcaug	gccacacacc	auguacaggu	ucuggugcuu	aggaguggac	aucuuuggga	480
ccgagggguu	uucugccuuc	cuaccauguc	accagaguug	ugcuaauaca	cagagagcuu	540
caggggauga	gaucugccau	ucauugagca	ccuucugugc	ggcagacagu	guuaggcaug	600
cuggaagaug	gacugcccuc	caauggugug	ccccgaucua	cagccccagg	uggaauaccc	660
aaccagaga	agaagacgaa	cugugagacc	cagugcccaa	auccccagag	ccucagcuca	720
ggcccucuga	cccagaaaca	gaauggccuu	cagaccacag	aggcuaaaag	agaugcuaag	780
agaaugccug	caaaagaagu	caccauaau	guaacagaua	gcauccaaca	gauggacaga	840
agucgaagaa	ucacaaagaa	cugugucaac	uagcagagag	uccaagcaga	agggcagaug	900
gacuucuuca	guguccuua	cggcacugga	ucccaucaa	gaaccuugaa	gaaguggcug	960
ccccuugcug	gaccugaauu	cuacugaguc	ccuggcaaga	cugucuuaac	uggcagcaaa	1020
cugcugccug	auuuguuggg	accuucugag	ccuucuaacu	aucauguaaa	uguauuggca	1080
cagugcuuac	auauguuaau	aaacugcaaa	ugugcaguuc	aguugucuc	uuugcaacuc	1140
cuguaauacg	gucuggugua	aaaguaguga	guuaaagcua	caggucaguu	uaugaaacag	1200
aaaaguagga	augcauuuuc	ugggugaaaag	agucacaccu	uagugcuaua	acucuccugc	1260
ccaugauagu	guauucuguu	ucaggcaagc	uuauucuuuc	cuucuuucau	uuuaauauu	1320
gucauuacaa	aucuuaccag	guucacuuaa	aagcuggcuu	ucauccaacu	cuaaacccac	1380
auauugaaaa	aaucaaggua	caggaaaacu	ccuuguuauc	cuuguuuccu	uagcuuggua	1440
ugagacagau	cggauccagu	uucccaugca	ccaacccacu	gcccaggca	ugucuuggg	1500
aggugucugu	gaagcaguca	uaccugcucc	ucaucugccu	ggaaaguccu	ccuauuccag	1560
uguccauguu	ggccuccagu	ccuuaauguc	accaugcuug	uggccaaugc	auccaaauaa	1620
ggauaccccu	cagggcucag	cuagacauug	caauuuugca	uagcuuucca	guucccuug	1680
cuugucuuuc	ugacugucuu	cccucucuau	cggggucacu	ugcaauuguu	aaucaaagau	1740
ugaacacugc	guaggagagg	gagaugaucc	agagacaugu	ggcagcaggc	auggcuuccc	1800
cuuggccucu	cuguacacug	ccccaggacu	gucauuuug	caucugcaaa	ggaaucacuu	1860
uagaaagcca	gcaccugguu	gauguguaau	cauacugaca	uuagauugau	gugcacugca	1920
uuagaaauga	gguagcugac	acagaaaaag	gauguuuuga	uaggaauaa	uuucuaguau	1980
gucuugaaac	auguucacu	ggaaguauuu	uccuccaaag	uaauguagca	ugauuuuuc	2040
aggauuguua	acaugccugg	gauugggaaa	gauaggacua	aaguugugcc	aaacuauauc	2100
aauaauucc	auguuuagca	gaaauaggca	gccuauuggu	guuauguua	uguaacauag	2160
uccagagaac	ugacaugcag	gucaaaaguc	agauacgcaa	ccuccuuauc	ugcuaacucu	2220
guuauucuu	aaacacaacg	uggguagugu	cauuuuuccu	uccuuccuuc	cauuggcaga	2280
uuguauauuu	auucacaaaa	cauuaaaugu	ccauccugug	ccagguacua	ugcagauguu	2340
gagggauuu	gggucugguu	agucgugacu	aucuauccug	aaucuaacag	ugacuucua	2400
acuaggagac	ugaauuagac	ccuuaaggua	uagugugugu	ugcaaaucac	ucugcaaugg	2460
aaacuuuuau	auucagggua	gguuuguguc	uuaaacuagg	uguucuaauc	aauguacaag	2520
acuuuaccu	acacgcaacu	auaguuuuuc	uaaaccuuc	ucauuuugug	auucuuugag	2580
aaagggcuuu	uaggaacuuu	auguucuaaa	aaauguuuuu	aacaauaaua	agauaaaaga	2640
aaaaccugug	auucauauu	ccccacuggc	auuacucagc	aggagcccc	agcugccaaa	2700

-continued

gguuggcagu gauccugcaa guucaagggc uuuuucuccc ugagggaugug cuuuguggcu 2760
ucucuuuaca gcuuuguuuc ugcaucaguu cacugcugca uguuguuugg aaauuauacac 2820
cuuaagaaag ugucucuguu uuauauagaa acacuuucuc acuuacaggg gagaaggaaa 2880
ugcagggcac augaucuggc ccuccccaga acaaucugga uuucacggag acagcaacca 2940
gaaguuaaac caugugacua aaaaugcauc ugguacuuiu uucauguaug uaugagacag 3000
aaacuaaucc uuacuauccu auuaggauac cacuuuucau ugcaaaguuu gugucaauaa 3060
agucauuauu uuuaaaca 3079

<210> SEQ ID NO 17
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Gly Cys Gly Gly Ser Arg Ala Asp Ala Ile Glu Pro Arg Tyr Tyr
1 5 10 15
Glu Ser Trp Thr Arg Glu Thr Glu Ser Thr Trp Leu Thr Tyr Thr Asp
20 25 30
Ser Asp Ala Pro Pro Ser Ala Ala Ala Pro Asp Ser Gly Pro Glu Ala
35 40 45
Gly Gly Leu His Ser Gly Met Leu Glu Asp Gly Leu Pro Ser Asn Gly
50 55 60
Val Pro Arg Ser Thr Ala Pro Gly Gly Ile Pro Asn Pro Glu Lys Lys
65 70 75 80
Thr Asn Cys Glu Thr Gln Cys Pro Asn Pro Gln Ser Leu Ser Ser Gly
85 90 95
Pro Leu Thr Gln Lys Gln Asn Gly Leu Gln Thr Thr Glu Ala Lys Arg
100 105 110
Asp Ala Lys Arg Met Pro Ala Lys Glu Val Thr Ile Asn Val Thr Asp
115 120 125
Ser Ile Gln Gln Met Asp Arg Ser Arg Arg Ile Thr Lys Asn Cys Val
130 135 140
Asn
145

<210> SEQ ID NO 18
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Gly Cys Gly Gly Ser Arg Ala Asp Ala Ile Glu Pro Arg Tyr Tyr
1 5 10 15
Glu Ser Trp Thr Arg Glu Thr Glu Ser Thr Trp Leu Thr Tyr Thr Asp
20 25 30
Ser Asp Ala Pro Pro Ser Ala Ala Ala Pro Asp Ser Gly Pro Glu Ala
35 40 45
Gly Gly Leu His Ser Gly
50

<210> SEQ ID NO 19
<211> LENGTH: 180
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Asn Cys Val Asn
180

<400> SEQUENCE: 20

Leu Gln Lys Val Gly His Pro Asn His
65 70

<400> SEQUENCE: 21

Gly Gly Leu His Ser Val Leu Glu Ala Glu Lys Ser Lys Ile Lys Ala

-continued

50	55	60	
Pro Thr Asp Ser Val	Ser Asp Glu Gly Leu Phe Ser Ala Ser Lys Met		
65	70	75	80
Ala Pro Leu Ala Val	Phe Ser His Gly Met Leu Glu Asp Gly Leu Pro		
	85	90	95
Ser Asn Gly Val Pro Arg Ser Thr	Ala Pro Gly Gly Ile Pro Asn Pro		
	100	105	110
Glu Lys Lys Thr Asn Cys Glu Thr	Gln Cys Pro Asn Pro Gln Ser Leu		
	115	120	125
Ser Ser Gly Pro Leu Thr	Gln Lys Gln Asn Gly Leu Gln Thr Thr Glu		
	130	135	140
Val Leu Leu Pro Ser			
145			
<210> SEQ ID NO 22			
<211> LENGTH: 80			
<212> TYPE: PRT			
<213> ORGANISM: Homo sapiens			
<400> SEQUENCE: 22			
Met Gly Cys Gly Gly Ser Arg Ala Asp	Ala Ile Glu Pro Arg Tyr Tyr		
1	5	10	15
Glu Ser Trp Thr Arg Glu Thr Glu Ser	Thr Trp Leu Thr Tyr Thr Asp		
	20	25	30
Ser Asp Ala Pro Pro Ser Ala Ala Ala	Pro Asp Ser Gly Pro Glu Ala		
	35	40	45
Gly Gly Leu His Ser Ala His Tyr Pro	Leu Ala Phe Ala Leu Ala Trp		
	50	55	60
Arg Asp Asn Ser Leu Gly Ala Leu Leu	Val Gln Glu Gly Leu Cys Arg		
65	70	75	80
<210> SEQ ID NO 23			
<211> LENGTH: 20			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<400> SEQUENCE: 23			
catctgttgg atgctatctg			20
<210> SEQ ID NO 24			
<211> LENGTH: 20			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<400> SEQUENCE: 24			
tggactctct gctagttgac			20
<210> SEQ ID NO 25			
<211> LENGTH: 20			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			
<400> SEQUENCE: 25			
tcggtgtagt tgatcttctc			20
<210> SEQ ID NO 26			
<211> LENGTH: 20			
<212> TYPE: DNA			
<213> ORGANISM: Homo sapiens			

-continued

<hr/>		
<400> SEQUENCE: 26		
accagagaa gaagacgaac		20
<210> SEQ ID NO 27		
<211> LENGTH: 20		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 27		
agaaacagaa tggccttcag		20
<210> SEQ ID NO 28		
<211> LENGTH: 20		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 28		
gccctctgac ccagaaacag		20
<210> SEQ ID NO 29		
<211> LENGTH: 23		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 29		
cttttgcagg cattctctta gca		23
<210> SEQ ID NO 30		
<211> LENGTH: 20		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 30		
tcttcgatgc caacaaggac		20
<210> SEQ ID NO 31		
<211> LENGTH: 19		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 31		
gcatcacgtc ctccgtcac		19
<210> SEQ ID NO 32		
<211> LENGTH: 25		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 32		
caggcattct cttagcatct ctttt		25
<210> SEQ ID NO 33		
<211> LENGTH: 30		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 33		
ctctttttagc ctctgtggtc tgaaggccat		30
<210> SEQ ID NO 34		
<211> LENGTH: 24		
<212> TYPE: DNA		
<213> ORGANISM: Homo sapiens		

-continued

<400> SEQUENCE: 34	
ttcagcttga ccctcaacac caac	24
<210> SEQ ID NO 35	
<211> LENGTH: 18	
<212> TYPE: PRT	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 35	
Asp Ala Ile Glu Pro Arg Tyr Tyr Glu Ser Trp Thr Arg Glu Thr Glu	
1 5 10 15	
Ser Thr	
<210> SEQ ID NO 36	
<211> LENGTH: 14	
<212> TYPE: PRT	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 36	
Asp Ser Ile Gln Gln Met Asp Arg Ser Arg Arg Ile Thr Lys	
1 5 10	
<210> SEQ ID NO 37	
<211> LENGTH: 25	
<212> TYPE: PRT	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 37	
Arg Ala Asp Ala Ile Glu Pro Arg Tyr Tyr Glu Ser Trp Thr Arg Glu	
1 5 10 15	
Thr Glu Ser Thr Trp Leu Thr Tyr Thr	
20 25	
<210> SEQ ID NO 38	
<211> LENGTH: 34	
<212> TYPE: DNA	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 38	
gtgcggtacc aagcttccgc ggcgaggag gatg	34
<210> SEQ ID NO 39	
<211> LENGTH: 32	
<212> TYPE: DNA	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 39	
cggggtaccg ttgacacagt tctttgtgat tc	32

- What is claimed is:
1. A method for characterizing acute myelogenous leukemia (AML) in a patient with AML, comprising assaying for overexpression of one or more BAALC transcripts in cells obtained from the patient,
wherein the BAALC transcripts are selected from the group consisting of transcripts identified as 1-8, 1-6-8, 1-5-6-8, 1-4-5-6-8, 1-5-6-7-8, 1-2-6-8, 1-2-5-6-8, and 1-2-3-6-8; and
wherein overexpression of one or more of the BAALC transcripts in cells of the patient indicates that the patient has an aggressive form of AML.
2. The method of claim 1 wherein overexpression of one or more of the BAALC transcripts in the patient's cells is assayed using a reverse-transcriptase polymerase chain reaction (RT-PCR).
3. The method of claim 2 wherein the RT-PCR employs a primer set selected from the group consisting of:
i) a forward primer having a sequence that is identical to a sequence in the sense strand of exon 6 of the BAALC gene and a reverse primer having a sequence that is complementary to a sequence in the sense strand of exon 8 of the BAALC gene,

61

- ii) a forward primer having a sequence that is identical to a sequence in the sense strand of exon 8 of the BAALC gene and a reverse primer that is complementary to a sequence in the sense strand of exon 8 of the BAALC gene, and
 - iii) a forward primer having a sequence that is identical to a sequence in the sense strand of exon 1 of the BAALC gene and a reverse primer that is complementary to a sequence in the sense strand of exon 1 of the BAALC gene.
- 4.** The method of claim **2** wherein the RT-PCR is real-time RT-PCR.
- 5.** The method of claim **4** wherein the real-time RT-PCR employs a probe that is complementary to a sequence within the product of the real-time RT-PCR, and wherein the probe has a reporter dye on end thereof and a quencher dye on another end thereof.
- 6.** The method of claim **1** wherein the cells are blood cells of the patient.
- 7.** The method of claim **1** wherein the AML patient has normal cytogenetics.
- 8.** A method for characterizing chronic myelogenous leukemia (CML) in a patient with CML, comprising assaying for overexpression of one or more BAALC transcripts in cells obtained from the patient,

62

- wherein the BAALC transcripts are selected from the group consisting of transcripts identified as 1-8, 1-6-8, 1-5-6-8, 1-4-5-6-8, 1-5-6-7-8, 1-2-6-8, 1-2-5-6-8, and 1-2-3-6-8; and
- 5 wherein overexpression of one or more of the BAALC transcripts in cells of the patient indicates that the patient is in blast crises.
- 9.** The method of claim **8** wherein the assay comprises determining the levels of one or more of the BAALC transcripts in leukocytes obtained from the patient.
- 10 **10.** The method of claim **2** wherein the RT-PCR employs a primer set selected from the group consisting of:
- i) a forward primer whose sequence comprises SEQ ID NO. 26 and a reverse primer whose sequence comprises SEQ ID NO. 23;
 - 15 ii) a forward primer whose sequence comprises SEQ ID NO. 27 and a reverse primer whose sequence comprises SEQ ID NO. 23; and
 - iii) a forward primer whose sequence comprises SEQ ID NO. 28 and a reverse primer whose sequence comprises SEQ ID NO. 29.
- 20 **11.** The method of claim **5** wherein the probe is selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:33.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,455,995 B2
APPLICATION NO. : 10/293239
DATED : November 25, 2008
INVENTOR(S) : Tanner et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b)
by 1267 days.

Signed and Sealed this
Eleventh Day of January, 2011

A handwritten signature in black ink, reading "David J. Kappos". The signature is written in a cursive, flowing style with a large initial "D" and "K".

David J. Kappos
Director of the United States Patent and Trademark Office